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ERRATA AND AUTHORS' EMENDATIONS

- Page 43, lines 27 and 28, hyphens should be inserted after "Small" in both cases and after "smooth."
- Page 43, fourteenth line from bottom, "(dO)" should be "(d)."
- Page 45, legend for Figure 3, "large" in both cases should be "Large."
- Page 258, seventh line from bottom, "×" after 2.269 should be "X."
- Page 261, paragraph 3, line 2, omit "were grouped together, and plots."
- Page 265, Table 7, last column, fraction line (shilling designation) should be omitted after "Diff." in the heading.
- Page 267, fifth line from bottom, the first "+0.035" should be "+0.373."
- Page 358, Fig. 2, "Terminal" should be omitted from legends for I and J.
- Page 516, line 32, "Mellon's" should be "Millon's."
- Page 517, line 30, "28°" should be "23°."
- Page 608, Table 2, "Trioxymethylene" should be "Trioxymethylene."
- Page 625, line 17, "nelanosae" should be "melanose."
- Page 648, line 7, sentence should be "The fungus which was isolated from Chinese cabbage and from rutabaga infected cabbage."
- Page 663, line 5, "Elliot" should be "Elliott." Throughout the article "*fumbriatum*" should be "*fumbriata*."
- Page 741, third line from bottom; page 742, lines 10 and 16: "(0.75 mol.)," "(0.8 mol.)," "(0.9 mol.)," respectively, apply only to the divalent precipitants and should be doubled for the monovalent.
- Page 742, line 1, Reference (19) is: Vogel, A., and Reischauer, C. 1859. In *Jahresber. Pharm.*, v. 11, p. 3
- Page 770, line 5, "*Aphanobacter*" should be "*Aplanobacter*."
- Page 782, line 7, "length" should be "index."
- Page 943, line 4 below tabulation, add "em. Wr." after "Sacc."
- Page 947, seventh line from bottom, "inconidiis" should be "in conidiis."
- Page 948, second line from bottom, add "I" after "forma."
- Page 993, in title of author, "Assistant" should be "Associate."
- Page 1028, heading "Infected Soil" should be "Infested Soil."
- Page 1075, Table 1, Lot 2, for sheep No. 393-961, under "Tensile Strength," "2,904" should be "2,094."
- Table 2, first part, for the average value of "Average elongation," ".003" should be ".0038."
- Table 2, second part, for sheep No. 541, under "Young's modulus," "9,54" should be "9,540."
- Page 1085, Table 5, Lot 2, for sheep No. 492, under "Tensile Strength," "176,190" should be "176,100."
- Page 1089, Literature Cited No. (3), "life weight" should be "live weight."
- Page 1091, in title, "W. & C." should be "Schlecht."
- Page 1146, line 29 and Literature Cited No. (27), "Jochinai" should be "Tochinai."
- Page 1148, in last column, "20 hours^b" should be "20 hours^c."
- Page 1150, line 5, add "(see fig. 1)" after "flagging" to replace same expression in line 3 of last paragraph.



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No. 1

EXPERIMENTS WITH CERTAIN ARSENATES AS SOIL INSECTICIDES¹

By B. R. LEACH

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INTRODUCTION

Three years ago a casual experiment, conducted in connection with certain other investigations of insecticides for the treatment of soil, demonstrated the fact that the larva of *Popillia japonica* Newm. dies when it feeds in soil containing a sufficient amount of certain arsenates. The constant ingestion of soil by the larva in the course of its feeding is conducive to rapid toxic action under the conditions referred to. The result of this experiment was of interest in that it offered a possible answer to certain problems in connection with the prevention of spread of the Japanese beetle.² Subsequent experimental work was therefore carried out at the Japanese Beetle Laboratory, Rivoton, N. J., for the purpose of ascertaining the effect of various arsenates upon the soil, plant, and insect.³

THE ARSENATES EMPLOYED

Acid lead arsenate (PbHASO₄)

	Per cent
Total arsenic oxide (As ₂ O ₅)-----	31. 97
Lead oxide (PbO)-----	63. 72
Water of constitution and undetermined-----	4. 31
Total-----	100. 00
Soluble arsenic oxide (As ₂ O ₅)-----	4. 05

Basic lead arsenate (Pb₄(PbOH) (AsO₄)₃H₂O)

Total lead oxide (PbO)-----	74. 22
Total arsenic oxide (As ₂ O ₅)-----	23. 54
Water-soluble arsenic oxide (As ₂ O ₅)-----	. 03
Water-soluble arsenic oxide equivalent to metallic arsenic (As)-----	. 02

¹ Received for publication Dec. 4, 1925; issued July, 1926.

² The Japanese beetle spends the major portion of its annual life cycle as a larva or grub in the soil, transforming to the beetle stage in midsummer and depositing eggs in the soil for the larvae of the succeeding generation.

³ The idea of applying arsenic to soil to kill insects present apparently was first conceived in the course of experiments by the Cornwall (England) County Council in 1899. A brief account of those experiments is given in the following report: HAWK, W. WIREWORMS. Cornwall County Council, Ann. Rpt. Agr. Expts. (1898) 3: 45-48. 1899. More recently, experiments with white arsenic (arsenious acid) have been made in Queensland, Australia, in connection with the control of grubs attacking the roots of sugar cane. See JARVIS, E. ON THE VALUE OF POISON BAIT FOR CONTROLLING CANE GRUBS. Queensland Bur. Sugar Expt. Stas. Div. Ent. Bul. 4, 14 p., illus. 1916.

⁴ Analyses made by Insecticide and Fungicide Board, Bureau of Chemistry, United States Department of Agriculture.

<i>Magnesium arsenate</i>	Per cent
Total arsenic oxide (As_2O_5)	44.33
Total arsenic oxide equivalent to $\text{Mg}_3(\text{AsO}_4)_2$	67.65
Water-soluble arsenic oxide ($(\text{AsO}_4)_2$)	1.03
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	.67
Total arsenic oxide equivalent to magnesium arsenate	67.65
Magnesium oxide in excess of arsenic oxide (MgO)	8.43
Calcium oxide (CaO)	1.98
Oxides of iron and aluminum (R_2O_3)	1.20
Acid-insoluble ash (silicious material)	.68
Water of hydration (CO_2), organic matter, etc. (by diff.)	20.06

Sample is a commercial grade of magnesium arsenate containing a small amount of casein.

<i>Zinc arsenate</i> ($\text{Zn}_3(\text{AsO}_4)_2$)	Per cent
Total zinc oxide (ZnO)	43.26
Total arsenic oxide (As_2O_5)	38.35
Total arsenic oxide equivalent to zinc arsenate ($\text{Zn}_3(\text{AsO}_4)_2$)	79.07
Water-soluble arsenic as arsenic oxide (As_2O_5)	.60
Water-soluble arsenic as arsenic oxide equivalent to metallic arsenic (As)	.39

<i>Hypothetical combinations</i>	
Zinc arsenate ($\text{Zn}_3(\text{AsO}_4)_2$)	79.07
Zinc oxide (ZnO) in excess of arsenic oxide	2.54
Loss on ignition	16.58
	<u>98.19</u>

<i>Copper arsenate</i> ($\text{Cu}_3(\text{AsO}_4)_2$)	
Arsenic oxide (As_2O_5)	46.47
Copper oxide (CuO)	37.92
Sodium oxide (Na_2O)	4.30
Iron oxide (Fe_2O_3)	.22
Loss on ignition	9.74
Water-soluble arsenic as arsenic oxide (As_2O_5)	3.10
Water-soluble arsenic as arsenic oxide equivalent to metallic arsenic (As)	2.02

<i>Hypothetical combinations</i>	
Sodium arsenate (Na_3AsO_4)	9.61
Tri-copper arsenate ($\text{Cu}_3(\text{AsO}_4)_2$)	55.54
Di-copper arsenate (CuHAsO_4)	24.63
Water of constitution, etc. (diff.)	10.22
	<u>100.00</u>

Sample is essentially tri-copper arsenate, di-copper arsenate, sodium arsenate, and water of constitution.

<i>Calcium arsenate</i> ($\text{Ca}_3(\text{AsO}_4)_2$)	Per cent
Total arsenic oxide (As_2O_5)	40.83
Total arsenic oxide equivalent to tri-calcium arsenate ($\text{Ca}_3(\text{AsO}_4)_2$)	70.70
Water-soluble arsenic oxide (As_2O_5)	3.63
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	2.37

<i>Ferric arsenate</i> (FeAsO_4)	
Ferric oxide (Fe_2O_3)	33.22
Total arsenic oxide (As_2O_5)	40.56
Total arsenic oxide equivalent to ferric arsenate (FeAsO_4)	68.73
Water-soluble arsenic oxide (As_2O_5)	.17
Water-soluble arsenic oxide equivalent to metallic arsenic (As)	.11

No ferrous iron detected.

Hypothetical combinations		Per cent
Ferric arsenate (FeAsO_4)	-----	68.73
Ferric oxide (Fe_2O_3) in excess to combine with As_2O_5	-----	5.04
Loss on ignition (water of constitution and moisture)	-----	25.36
		99.13

TOXICITY OF ARSENATES TO LARVA IN THE SOIL

The various arsenates mentioned above were tested to determine their toxicity to the larva of the Japanese beetle. This work was done during the late winter under greenhouse conditions, the soil temperature averaging about 60° F. In each case the specific quantity of the arsenate was thoroughly mixed with the required amount of soil, and the mixture was placed in 4-inch flowerpots on the greenhouse bench, each test embracing 5 pots. The pots of soil were then moistened, and 24 hours later they were infested with 4 larvae per pot. The results of these tests are given in Table 1.

TABLE 1.—Toxicity to third instar larvae of the Japanese beetle of various arsenates when mixed with soil (soil temperature,^a 60° F.)

Compound	Amount per 4-inch pot	Equivalent amount per acre	Days required to kill all larvae
	Gms.	Lbs.	
Acid lead arsenate	0.275	500	40
Do.	.55	1,000	18
Do.	.825	1,500	10
Do.	1.10	2,000	7
Do.	1.65	3,000	5
Basic lead arsenate	0.55-2.75	1,000-5,000	(^b)
Zinc arsenate	.55-2.75	1,000-5,000	(^c)
Calcium arsenate	.55-2.75	1,000-5,000	(^d)
Copper arsenate	.55-2.75	1,000-5,000	(^e)
Magnesium arsenate	.55-2.75	1,000-5,000	(^b)
Ferric arsenate	.55-2.75	1,000-5,000	(^b)

^a The speed of kill for a definite concentration of arsenate in soil depends upon the soil temperature; the larva is relatively inactive below 50° F.

^b Larvae not killed.

^c Killed somewhat more slowly than acid lead arsenate.

^d About the same as acid lead arsenate.

^e Same as zinc arsenate.

The basic lead arsenate, the magnesium arsenate, and the ferric arsenate were not toxic to the larvae. The zinc arsenate and the copper arsenate were slower in killing the larvae than acid lead arsenate and calcium arsenate. Summarizing the results, the experiments indicate, from the standpoint of larval mortality, that acid lead arsenate is the best arsenate for killing larvae in the soil.

OUTDOOR EXPERIMENTS WITH ACID LEAD ARSENATE

EXPERIMENT NO. 1

On April 21, 1922, four wooden frames, 21 inches wide, 36 inches long, and 8 inches deep, with a 16-mesh wire-screen bottom, were sunk in the soil outdoors, the top of the frame being level with the surface of the soil. Ordinary potting soil was then placed in each frame to a depth of 4 inches and infested with 100 third-instar larvae of the Japanese beetle per frame. Frames Nos. 1, 2, and 3 were then

filled with soil containing acid lead arsenate at the rate of 1,000, 2,000, and 3,000 pounds per acre, respectively. Frame No. 4 was filled with ordinary soil containing no arsenate of lead.

At this time (April 21) the soil temperature was low (40° F.), and the larvae were inactive, but by May 2 the soil had warmed up considerably and the larvae were moving into the upper 4 inches of arsenated soil. The examinations of the soil in the various frames made at subsequent intervals are given in Table 2.

TABLE 2.—*Mortality of third-instar Japanese beetle larvae in outdoor soil arsenated to a depth of 4 inches, 100 larvae being used in each test (experiment begun April 21, 1922)*

Date of examination	1,000 pounds		2,000 pounds		3,000 pounds		Control	
	Number alive in upper 4 inches	Number alive in lower 4 inches	Number alive in upper 4 inches	Number alive in lower 4 inches	Number alive in upper 4 inches	Number alive in lower 4 inches	Number alive in upper 4 inches	Number alive in lower 4 inches
May 8.....	60	20	32	28	24	12	48	52
May 15.....	8	24	8	24	0	36	40	56
May 22.....	4	16	6	4	4	6	46	46
May 31.....	4	10	2	2	2	4	41	43
June 12.....	0	0	0	0	0	0	^a 20	^b 17

^a Eleven others had pupated.

^b Twenty-four others had pupated.

In the untreated soil the larvae remained normal and were pupating by June 12, while in the arsenated soil the larvae were succumbing to the poison and by June 12 all were dead.

EXPERIMENT NO. 2

Wire-screen cages, 16-mesh wire, 5 feet square by 5 feet in height, were employed. These were set up in a row outdoors on July 1, 1922, with the cage baseboards sunk in the ground. The soil in the individual cages was treated on July 2, 1922, as shown in Table 3.

TABLE 3.—*Results of experiment No. 2 with larvae of the Japanese beetle in arsenated soil*

Cage No.	Treatment of soil in cage made July 2, 1922	Number of larvae found Sept. 1	
		In untreated soil of cage	In treated soil of cage
1	Control, soil not treated.....	475	-----
2	Soil received acid lead arsenate ^a at rate of 1,000 pounds per acre.....	-----	0
3	Soil received acid lead arsenate at rate of 2,000 pounds per acre.....	-----	0
4	One-half soil area of cage received acid lead arsenate at rate of 1,000 pounds per acre; remainder of soil area untreated ^b	268	0
5	Same as 4, but arsenate employed at rate of 2,000 pounds per acre.....	231	0

^a Spread evenly over surface of the soil and worked in to a depth of 4 inches with rake and hoe.

^b Treated and untreated portions separated by a board sunk into the soil.

Twelve hundred and fifty Japanese beetles were placed in each cage, in lots of 250 at intervals of 4 days, beginning July 5, and smartweed was supplied as food. The beetles deposited eggs indiscriminately in both the treated and the untreated soil, but the larvae coming from those deposited in the treated soil died very shortly after hatching.

EFFECT OF ARSENATED SOIL ON PLANTS

The effect of arsenated soil on plants depends upon the nature of the arsenate. The experiments at the Japanese Beetle Laboratory indicate that the arsenates nontoxic to the larvae are nontoxic to plants. The basic arsenate of lead, the magnesium arsenate, and the ferric arsenate are typical examples in this respect. When mixed with soil at the rate of 3,000 pounds per acre, these arsenates did not seem to inhibit the growth of the common garden bean to the slightest extent.

The arsenates which proved toxic to the larvae also proved toxic to plants, although there were some outstanding exceptions. The arsenates of copper, zinc, and calcium were decidedly toxic to plants, only one plant (*Pteris wimsetti*) withstanding them to any appreciable extent. It was, however, with acid arsenate of lead that the outstanding instances of plant resistance to a larva-killing arsenate were encountered. The presence in the soil of relatively large amounts of this compound is tolerated to a surprising extent by nasturtium, snapdragon, *Pteris wimsetti*, *Vinca minor*, *Clematis paniculata*, and certain of the grasses. These plants were equal in growth to the controls, and in several instances were superior to them, notably in the case of *Pteris wimsetti*. Nasturtium and snapdragon bloomed normally when grown in soil containing acid lead arsenate at the rate of 2,000 pounds per acre. On the other hand, certain plants such as *Salvia splendens*, *Calendula*, and strawberry made no growth in soil so treated, while there are numerous plants which have some resistance to this arsenate, the degree of resistance varying with the species. In general, the results of the experiments indicate that the plant phase of the problem offers much of fundamental interest to the plant physiologist.

SOIL ACTION UPON ACID LEAD ARSENATE⁵

Acid lead arsenate undergoes chemical change when mixed with moist soil, the nature of the change being indicated by the reaction of the plant and insect. In all probability the compound is acted upon by the soil solution, with the consequent formation of insoluble basic arsenates and soluble arsenates, with the latter ultimately leaching away or being absorbed or possibly rendered impotent by buffer reactions. The presence of soluble arsenic is indicated by the stunting of plant growth, while the formation of insoluble basic arsenates is indicated by a certain degree of loss of toxicity toward the larvae.

⁵ For the most complete account of the action of the soil solution upon acid arsenate of lead see the following: STEWART, J. SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 1. Soil Sci. 14: 111-118. 1922. — and SMITH, E. S. SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 2. Soil Sci. 14: 119-126, illus. 1922.

This decrease of toxicity toward the larvae, however, depends upon the amount of acid lead arsenate applied to the soil, as indicated in Table 4.

TABLE 4.—*The decrease in toxicity to the larvae of the Japanese beetle caused by the action of the soil solution upon the arsenate of lead applied*

Pounds per acre	Number of days required to kill larvae introduced into soil which had been mixed with the arsenate for various periods of time			
	Imme- diately after mixing	After being mixed 1 year	After being mixed 2 years	After being mixed 3 years
500	40	(*)	(*)	(*)
1,000	18	40	40	40
1,500	10	(*)	(*)	(*)
2,000	7	10	10	10

* No data.

It will be noted that there is a certain loss in toxicity to the larvae due to the breakdown of a part of the arsenate in the soil, whereupon the degree of toxicity persisting tends to remain constant. In practice, therefore, it would seem advisable either to apply a sufficient initial quantity of arsenate to allow for the loss in toxicity of a portion, or to reinforce a small initial application with a subsequent supplementary one. How long the toxicity to the larvae of the larger applications will endure, and the various factors influencing this are not known, but experiments are now under way to obtain data along these lines.

THE PRACTICAL USE OF ACID LEAD ARSENATE AS A SOIL INSECTICIDE ⁶

The experimental data outlined above have been applied to the solution of certain problems, and a considerable quantity of acid lead arsenate is now being used in the quarantined area. At present acid arsenate is being used extensively for freeing soil of larval infestation and maintaining it in that condition.

Large quantities of field-grown perennials are dug in the early fall by certain of the local nurseries after the beetle flight is over, potted in clean soil, and the pots plunged into cold frames the soil of which has been treated one month previously with acid lead arsenate at the rate of 1,500 pounds per acre ⁷ (3.5 pounds per square foot). The arsenate is spread evenly on the surface and worked in to a depth of 4 inches, the work being preferably done when the soil is on the dry side. Under the system just described this potted stock can be held for future shipment with no danger of infestation. The pots could

⁶ The foregoing account of this work was written in August, 1924. Since that time the writer, assisted by J. W. Lipp, of the Japanese Beetle Laboratory, has continued the work, paying especial attention to the use of acid lead arsenate as a poison for the larvae of the Japanese beetle in lawns and golf greens, which work has shown that many of the finer grasses, notably the bents, do remarkably well in poisoned soil while the grub is effectively controlled. Extensive grass plot tests are now under way along these lines. See LEACH, B. R., and LIPP, J. W. A METHOD OF GRUB-PROOFING TURF. Bul. Green Sec. U. S. Golf Assn. 6:34-39, illus. 1926.

⁷ The narrow paths between the cold frames are also treated.

not be placed in untreated soil without the ever-present danger of larvae working their way into the pots through the holes at the bottom.

Several of the local nurseries have also treated areas of ground of an acre or less with arsenate of lead in order to have available a piece of ground free of larvae for the purpose of heeling in deciduous shrubbery, iris, peonies, funkia, etc., during the shipping season. In treating larger areas of this sort the arsenate is applied to the plowed ground (1,500 pounds per acre) with a fertilizer drill and thoroughly worked over with disk and spiketooth harrows, the work being done when the soil is on the dry side and a month before the ground is to be used for the above purpose. Soil taken at random from areas so treated and placed in a flowerpot, within two weeks, gave 100 per cent mortality of larvae placed therein. Moreover, no larvae were found in the soil so treated 14 months later at the conclusion of the next year's beetle flight, indicating that the larvae emanating from eggs deposited therein quickly succumbed. Soil treatment of this sort is supervised by the quarantine officials, and thoroughness of application is insisted upon.

The ultimate effect of lead arsenate on soil so treated is not known at present, and it is urged that only the minimum area of ground necessary for the conduct of business be so treated.

EXPERIMENTS WITH COATED ARSENATE OF LEAD

In view of the chemical change which acid lead arsenate undergoes in the soil and the complications arising thereby in the use of this compound as a soil insecticide, the idea was conceived of coating the impalpable particles of the arsenate with materials which would prevent the chemical action of the soil solution upon the arsenate but which would be broken down by the gastric juices in the intestinal tract of the larvae, thereby exposing the arsenate of lead and permitting its toxic action.

Oils, fats, waxes, and various plant, animal, and mineral compounds of the type suitable for this purpose have been tested in this connection during the last three years, a method of coating suggested by P. A. van der Meulen of Rutgers College being employed. This method consists essentially in dissolving the coating material in some suitable solvent such as benzene or alcohol, stirring in the arsenate of lead, and evaporating to dryness. Perfect coatings can be obtained by this method, 100 grams of lead arsenate being coated with 5 to 15 grams of coating material. With a larger proportion of the coating material the final product is likely to be too sticky for use in soil.

The results of the experimental work with coated arsenate of lead to date have been negative; the coating materials have been destroyed by the soil action, thereby exposing the arsenate of lead to the soil action. Until recently it was thought that the destruction of the coat was entirely a chemical action, but it is rather far-fetched to attribute the loss of such a chemically inert coating material as paraffin to the action of the relatively dilute soil solution. In this connection tests made at this laboratory by W. E. Fleming indicate that certain soil organisms are responsible for the loss of the paraffin coating. It is therefore proposed to continue the work on the coating of arsenate of lead for use in soil, special attention being given to both the chemical and soil-biological aspects of the problem.

SUMMARY

The larva of the Japanese beetle, *Popillia japonica* Newm., dies when it feeds in soil containing a sufficient amount of certain arsenates. The compounds tested in this connection were basic and acid lead, magnesium, zinc, copper, calcium, and ferric arsenates. The basic lead, magnesium, and ferric arsenates were nontoxic to the larva. Acid lead arsenate proved the most satisfactory from the standpoint of larval toxicity. At a soil temperature of 60° F. the larva succumbs to the acid lead arsenate in a certain number of days when feeding in soil treated with the compound, the number of days required to kill the larva depending upon the concentration of arsenate. The arsenates nontoxic to the larva proved also to be nontoxic to plants. Of the arsenates lethal to the larva acid lead arsenate was least toxic to plants. Nasturtium, snapdragon, *Pteris wimsetti*, *Vinca minor*, *Clematis paniculata*, and *Poa annua*, grew normally in acid lead arsenate applied at the rate of 2,000 pounds per acre. Many plants showed considerable tolerance for the compound, while many others tested were severely checked in growth. Acid lead arsenate undergoes chemical change when mixed with moist soil, the tendency being toward the formation of basic arsenates. Acid lead arsenate applied at the rate of 1,500 pounds per acre is now being used to a considerable extent by nurseries in the area infested by the Japanese beetle as a means of freeing soil of the larva and keeping it free. The results of experiments in coating the particles of lead arsenate for the purpose of resisting soil action have been negative so far.

WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII IN 1921¹

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INTRODUCTION

The Mediterranean fruit fly (*Ceratitis capitata* Weidemann considered by many to be the most destructive insect pest of tropical and subtropical fruits, was first found in the Hawaiian Islands in 1910. Since then various methods of bringing it under control have been tried. Clean culture (the destruction of all host fruits spraying with poisoned baits, trapping, and covering fruits before infestation occurs, have all been thoroughly tested. No practicable method of control, for general Hawaiian conditions, has yet been discovered. However, under the peculiar conditions existing in the Kona coffee fields on the island of Hawaii, introduced parasites have so decreased the number of fruit flies that the coffee cherries are now nearly free from infestation.

In Hawaii, natural enemies accomplish a greater degree of control than any other agency. In a previous publication (7)² it was shown that the ant *Pheidole megacephala* Fab. may destroy from one third to four-fifths of the fruit-fly larvae developing in fruits which have fallen to the ground. Other natural enemies of minor importance occur; but the most effective natural control has been accomplished by four species of larval parasites which were introduced by the Territory of Hawaii. Two opiines, *Opius humilis* Silvestri from Africa and *Diachasma tryoni* Cameron from Australia were introduced in 1913; and one opiine, *Diachasma fullawayi* Silvestri, and one chalcid, *Tertastichus giffardianus* Silvestri, from Africa in 1914.

The Bureau of Entomology of the United States Department of Agriculture has kept careful records of the degree of parasitism attributable to each of the four species since their liberation in 1914. Records of the amount of infestation of different fruits by the fruit fly since 1916 have also been kept. These records have been made from almost daily collections of fruits from the vicinity of Honolulu, from which careful counts of parasitized and nonparasitized flies have been made. Such records are a good guide to the progress of each parasite under Hawaiian conditions, and give definite information on the number of flies destroyed by parasites each year.

RECORDS OF PARASITISM

The results of these observations for 1914 to 1920, inclusive, have been published (1, 2, 4, 6, 8, 9) annually, except those for 1919 and 1920, which were published in one paper.

¹ Received for publication Dec. 12, 1925; issued July, 1926.

² Reference is made by number (italic) to "Literature cited," p. 15.

The data in Table 5 show that since 1916 the yearly variation in parasitism has not been great, but that considerable variation occurs over a period of three years. The parasitism during 1916 was 33.2 per cent, increasing to 55.8 per cent in 1918; in 1919 it dropped to 3.2, and increased in 1921 to 56.4. Abundance of adult flies in the field is indicated by the numbers of maggots in the host fruits. In 1913 and 1914, before parasites had become established, *Ceratitis capitata* evidently reached its maximum abundance. No statistical records which might give the exact degree of infestation of different host fruits were compiled before the liberation of the parasites. However, numerous statements made to the writers by fruit growers, entomologists, and others whose observations were considered reliable, all definitely agree that maximum infestation occurred at that time, and also that there was a very noticeable decrease in infestation during the first three years after the liberation of parasites.

If records of the average number of maggots per fruit are made from a sufficiently large number of fruits each year, they will show the comparative abundance of adults in the field from year to year. Records of this kind were begun in Honolulu in 1916. Table 1 gives the average infestation of each fruit in 1921, together with the number of fruits and larvae under observation. During the year 33,646 fruits were collected, from which 677,575 larvae were taken, this collection being typical of the large numbers of fruits from which the records have been made each year.

TABLE 1.—Extent of infestation of host fruits by larvae of *Ceratitis capitata* in Hawaii in 1921

Host fruit	Number of fruits collected	Number of larvae emerging	Average number of larvae per fruit
Indian almond (<i>Terminalia catappa</i>)	30,968	325,865	10.5
lango (<i>Mangifera indica</i>)	1,525	5,961	3.9
coffee (<i>Coffea arabica</i>)	4,173	1,693	4
strawberry guava (<i>Psidium cattleianum</i>)	3,575	12,176	3.4
black myrobalan (<i>Terminalia chebula</i>)	12,924	91,048	7.0
French cherry (<i>Eugenia uniflora</i>)	16,901	31,546	1.9
Vest Indian medlar (<i>Mimusops elengi</i>)	4,058	26,737	6.6
apodilla (<i>Achras sapota</i>)	249	1,985	8.0
arambola (<i>Averrhoa carambola</i>)	222	222	1.0
loquat (<i>Eriobotrya japonica</i>)	701	3,168	4.5
Chinese orange (<i>Citrus japonica</i>)	49,118	127,947	2.6
Guava (<i>Psidium guajava</i>)	6,306	42,052	6.7
Orange (<i>Citrus aurantium</i>)	1,467	3,240	2.2
Bitter orange (<i>Citrus nobilis</i>)	1,065	3,290	3.1
Choronia emarginata	201	365	1.8
Choronia elliptica	193	280	1.5

The data in Tables 1 and 2 were obtained from host fruits gathered for the purpose of obtaining records of fruit-fly parasitism, and only ripe, well-infested fruits were collected. Edible host fruits picked for market are removed from the trees in the earliest stages of maturity, when they have been exposed to a minimum of fruit-fly attack and show very little, in some cases no, infestation. Tables 1 and 2, therefore, are not records of the infestation of fruits intended for commercial purposes.

Table 2, for purposes of comparison, gives the average number of maggots per fruit for each year from 1916 to 1921, inclusive, together with the average per fruit for the 6-year period. The extent of these observations is indicated by the fact that 788,826 fruits

were collected, from which 2,830,310 larvae were taken. A comparison of the average infestation per fruit in all species of hosts during each year, with the average infestation for the 6-year period shows that the proportion of hosts with more than average infestation steadily decreased from 1916 to 1920, and greatly increased in 1921. In 1916, 47.1 per cent of the total number of species of host fruits had a higher average number of larvae per fruit than the average for the 6-year period shown in the last column of Table 2. Similar comparisons for the five years which followed show that in 1917 40 per cent of the host fruits were over the 6-year average in infestation; in 1918, 35.3 per cent; in 1919, 33.3 per cent; in 1920 31.6 per cent; and that in 1921 the number increased to 68.8 per cent. The unusually high infestation of fruits during 1921 was accompanied by the greatest abundance of parasites since the liberation, resulting in a total parasitism for the year of 56.4 per cent.

TABLE 2.—Average number of *Ceratitis capitata* larvae per fruit in Honolulu for the period 1916 to 1921, inclusive, and for each year of the period

Host fruit	Average number of larvae per fruit						For 6-year period
	1916	1917	1918	1919	1920	1921	
Indian almond	9.5	8.0	9.9	8.4	5.5	10.5	
Mango	1.7	8.1	24.4	3.7	3.5	3.9	
Coffee	.5	.8	.6	.4	.6	.4	
Strawberry guava	1.6	2.0	1.3	1.2	1.2	3.4	
Black myrobalan	7.0	5.9	4.8	4.7	6.9	7.0	
French cherry	.8	1.0	1.0	.9	1.4	1.9	
Satin leaf (<i>Chrysophyllum olivaeforme</i>)	2.0	3.4	3.2	5.0	4.7		
Rose apple (<i>Eugenia jambos</i>)	5.5	8.8	6.6		11.5		
West Indian medlar	5.3	1.8	2.5		.4	6.6	
Sapodilla		4.7				8.0	
Carambola	1.3	1.6	.9	.05	.2	1.0	
Yellow oleander (<i>Thevetia neriiifolia</i>)	3.6	5.7	6.0	1.7	1.9		
Loquat		2.6	1.8	3.4		4.5	
Chinese orange	3.1	1.8	1.8	2.5	2.4	2.6	
Guava	6.8	4.5	8.5	9.8	7.2	6.7	
Orange				3.3	6.0	2.2	
Tangerine orange					.9	3.1	
Lime (<i>Citrus medica limetta</i>)					1.2		
Noronhia emarginata	1.7		1.1		.1	1.8	
Orchrosia elliptica	3.1	23.5				1.5	
Kamani (<i>Calophyllum inophyllum</i>)	3.3	2.4	2.4	6.0	4.9		
Peach (<i>Amygdalus persica</i>)	20.5	15.2	22.4	13.6	15.6		18
White sapote (<i>Casimiroa edulis</i>)		7.6					7
Wampi (<i>Clausena wampi</i>)		.2					

The data in Table 3 are recorded to show the ability of each species of parasite to attack its host in different species of fruits, and to indicate any preference on the part of each parasite for particular host fruits. *Opius humilis*, *Diachasma tryoni*, and *D. fullawayi*, all of which oviposit in the larvae of *Ceratitis capitata* from the outside through the skin of the fruit, are capable of parasitizing only those maggots which are very close to the outer surface, where they are within reach of the parasite's ovipositor. Consequently, the ability of these opiines to attack their host is much greater in small fruits and in those containing a large seed covered by a thin layer of pulp into which the maggots can not penetrate deeply. The ability of *Tetrastichus giffardianus* to parasitize *C. capitata* depends more upon the nature of the fruit than does that of the opiines. This parasite enters the fruit and searches out the host larvae in which to oviposit and a crack or other opening in the skin is necessary before it can reach its host.

TABLE 3.—Percentage of larval parasitism of *Ceratitis capitata* in Hawaii in 1921

Host fruit	Month	Number of larvae emerging during first 2 to 6 days	Percentage of parasitism				Total
			<i>Opius humilis</i>	<i>Diachasma tryoni</i>	<i>Diachasma fullawayi</i>	<i>Tetrastichus giffardianus</i>	
Lian almond	April	4,440	8.3	6.4	0.1	0.5	15.3
	May	15,676	19.4	58.7	1.3	1.8	81.2
	June	2,750	25.0	34.4	.8	6.6	66.8
	July	8,487	3.6	28.3	4.2	19.5	55.6
	August	12,976	5.9	21.9	4.2	39.7	71.7
	September	5,431	6.3	28.4	1.5	38.9	75.1
	October	3,904	3.1	31.9	.5	13.3	48.8
	November	4,253	3.4	26.3	1.2	20.4	51.3
	December	673	6.5	28.5	3.0	26.2	64.2
Mango	April	220	9.1	5.9	8.2	3.2	26.4
	May	22		9.1			9.1
	June	171	3.5	11.1	8.8	10.0	33.4
	July	325	2.2	22.2	9.2	6.5	40.1
Coffee	January	41	19.5	2.4	4.9		26.8
	February	400	12.3	7.7	19.7		39.7
Rawberry guava	do.	122	.8			.8	1.6
	March	230	11.3	12.2	13.9	1.3	38.7
	April	471	3.4	4.2	3.6	.2	11.4
	June	392	2.5	21.7	27.8	25.5	77.5
	July	400	3.0	34.5	24.0	21.2	52.7
	October	197	6.7	19.3	1.0	46.2	73.2
	February	1,878	.3	7.1	.5	15.3	23.2
	October	4,127	.7	10.4	7.7	.6	19.4
	November	2,051	1.6	15.3	12.9	4.8	34.6
Peach cherry	January	526	12.3	38.0	10.5	4.4	65.2
	February	1,199	5.5	36.5	28.2	12.9	83.1
	March	13	7.7		23.1	23.1	53.9
	June	262	3.8	44.7	31.7		80.2
	July	76	2.5	21.1	25.0	9.2	57.8
	September	811	1.2	19.8	36.7	3.6	61.3
	October	2,058	1.7	32.4	46.1	3.1	83.3
	November	1,483	4.6	19.2	17.8	3.5	45.1
	March	330			.3		.3
West Indian medlar	April	1,563	2.1		.7	.5	3.3
	May	168	5.4	6.4		1.2	13.0
	June	170	2.4	1.8	1.8	18.2	24.2
Arambola	July	44				40.9	40.9
	September	17		5.9		58.8	64.7
	October	73	6.9			64.4	71.3
Coquat	February	559	1.2	21.1	36.3	1.1	59.7
	January	69	1.5	10.1	4.4	7.2	23.2
Chinese orange	February	467	1.3	4.1	2.4	1.7	9.5
	March	636	5.8	1.4	4.4	3.3	14.9
	April	757	8.1	3.8	8.1	4.6	24.6
	May	994	5.8	17.3	3.9	1.9	28.9
	June	248	8.9	10.5	.8	20.1	40.3
	July	200	3.0	8.0	1.5	37.0	49.5
	August	33		6.1		18.2	24.3
	September	38	7.9	2.7		7.9	18.5
	October	15	13.3			13.3	26.6
	November	205	2.4	2.4	.5	8.3	13.6
	December	477	5.0	6.7	2.1	1.9	15.7
	January	61		1.6		22.9	24.5
	February	245		12.7	9.0	6.5	28.2
	March	558	1.6	11.8	1.6	37.3	52.3
	April	349	5.7	8.3	2.6	42.7	59.3
Orange	May	548	.4	14.2	5.5	37.0	57.1
	June	359	1.4	9.5	3.9	20.3	35.1
	July	138	8.0	10.9	5.1	31.1	55.1
	August	627	4.0	10.7	4.1	36.2	55.0
	September	112		24.1		4.5	28.6
	October	168		4.2	11.3	19.6	35.1
	November	1,168	2.3	1.0	1.1	68.8	73.2
	December	459	1.7	.4	2.0	65.8	69.9
	January	97	2.1	5.1		7.2	14.4
	February	96	1.0	7.3		15.6	23.9
	March	46				37.0	37.0
	April	6					
	May	27		3.7	3.7	14.8	22.2
	June	34	2.9		2.9	41.2	47.0
	July	7			14.3		14.3
	September	39	2.6	7.7	5.1	43.6	59.0
Mandarin orange	October	6				50.0	50.0
	May	31	6.4	22.6	3.2	9.7	41.9
	June	63	3.2	20.6		1.6	25.4
Voronhia emarginata	September	169	2.4	.6	1.2	12.4	16.6
	July	75		1.3			1.3

Table 3 clearly shows this variation in effectiveness in different fruits. Indian almond (*Terminalia catappa*) is a good example of fruit having a large seed and thin pulp. Its seed is from 1 to 2 inch long, about 1 inch wide, and $\frac{1}{2}$ to $\frac{3}{4}$ inch thick, and is surrounded by $\frac{1}{8}$ to $\frac{3}{8}$ inch of pulp, which is firm in texture and does not break easily when the fruit falls to the ground. It is usually well infested with maggots, and is available for collection about Honolulu nearly every month of the year. In this fruit, during 1921, the total parasitism by the opiine parasites was greater than that of *Tetrastichus giffardianus* for seven months and less for two months. Guava (*Psidium guajava*) and orange (*Citrus aurantium*) exemplify the large pulpy type of fruits in which *T. giffardianus* is more effective than the parasites which attack the maggots from the outside. When guavas ripen they fall off and often crack open upon impact with the ground, or there are holes in them made by birds or certain insects which allow *T. giffardianus* to enter freely and attack its host. Infested oranges, although they do not usually break open when they hit the ground, contain breathing holes made by fruit-fly larvae (through which *T. giffardianus* finds access to its host. In 1921 the parasitism of *Ceratitis capitata* by *T. giffardianus* in guavas was greater than the total parasitism by the three braconid parasites for 10 months and less for two months. In oranges, in 1921, it was greater for six months, the same for one month, and less for one month. The work of *T. giffardianus* is of special value because it attacks its host very readily in fruits where the maggots are inaccessible to the other three species, and particularly in guavas, which is the most abundant and widely distributed host of the fruit fly in Hawaii.

The percentages of parasitism shown in Table 3 indicate that a few parasites will attack their host in any fruit where the maggots are accessible, showing no great preference for maggots in one species of fruit over those in another. *Diachasma fullawayi*, however, has attacked the larvae in French cherry (*Eugenia uniflora*), strawberry guava (*Psidium cattleianum*), loquat (*Eriobotrya japonica*), and coffee (*Coffea arabica*), much more heavily than it has the larvae in other fruits.

Table 4, giving the percentages of parasitism by each species in all fruits under observation for each month in the year, shows the efficiency of each parasite during all seasons. In 1921 *Opius humilis* was the most effective species for one month, *Diachasma tryoni* for six months, and *Tetrastichus giffardianus* for five months. Similar tables previously published (4, 6, 8, 9) show a steady increase in the relative importance of *T. giffardianus*. In 1915 and 1916 it was neither first nor second in amount of parasitism during any month. In 1917 and 1918 it was first in no month, but was second in relative importance during four months of each year. In 1919 it was first for one month and second for five months. In 1920, it was first for two months and second for one month, whereas in 1921 it had increased to the point where it was the most valuable parasite of the four for five months and the second most valuable for one month. Much of the parasitism by this species has been in the more fleshy fruits, where the opiine parasites work with difficulty, so that its great increase during 1921 considerably increased the total parasitism of *Ceratitis capitata* for that year.

TABLE 4.—Total parasitism of *Ceratitis capitata* larvae from all fruits collected in Hawaii in 1921 (averaged by months)

Month	Number of larvae	Percentage of parasitism				Total
		<i>Opius humilis</i>	<i>Diachasma tryoni</i>	<i>Diachasma fullawayi</i>	<i>Tetrastichus giffardianus</i>	
January.....	794	9.6	26.9	7.6	6.2	50.3
February.....	4,966	2.7	15.7	13.3	9.5	41.5
March.....	1,813	4.0	5.7	4.0	13.9	27.6
April.....	7,806	6.6	4.8	1.6	2.8	15.8
May.....	17,466	17.8	54.3	1.6	2.9	76.6
June.....	4,449	16.8	27.9	5.6	10.5	60.8
July.....	9,752	3.6	27.3	5.3	19.5	55.7
August.....	13,636	5.8	21.4	4.1	39.4	70.7
September.....	6,617	5.5	26.2	5.8	33.2	70.7
October.....	10,548	2.0	22.6	12.4	7.4	44.4
November.....	9,160	3.0	18.9	6.5	20.1	48.5
December.....	1,609	4.7	14.0	2.4	30.3	51.4

The total parasitism by each species in all fruits as recorded in table 5 definitely shows the efficiency of each parasite and the total parasitism by all four species from 1915 to 1921, inclusive. *Opius humilis* has declined steadily in effectiveness since 1915, when it parasitized 31.5 per cent of *Ceratitis capitata* in all fruits until 1921, when its parasitism was only 7.6 per cent. From a position of first importance in 1915 and 1916, it has been reduced in numbers until it ranked third in effectiveness in 1920 and 1921. As previously recorded (3, 5), this decrease is the result of the certain destruction of *O. humilis* by either *Diachasma tryoni* or *Diachasma fullawayi* when they occur in the same host larva. The two species of *Diachasma* will probably never eliminate *O. humilis* from Hawaii, but they will doubtless decrease its numbers to such an extent that it will be the least effective of any of the four parasites, and a very small factor in the killing of *C. capitata*. It has been maintained as a result of previous investigations (3, 5) that *O. humilis*, being more prolific and capable of destroying a higher percentage of *C. capitata* under Hawaiian conditions than either species of *Diachasma*, would be more effective, working alone, than *O. humilis*, *D. tryoni*, and *D. fullawayi* combined. The steady decrease of *O. humilis*, from a place of first to one of very little importance, supports this contention.

TABLE 5.—Total parasitism of all larvae of *Ceratitis capitata* collected in Hawaii from 1915 to 1921, inclusive

Year	Number of larvae	Percentage of parasitism				Total
		<i>Opius humilis</i>	<i>Diachasma tryoni</i>	<i>Diachasma fullawayi</i>	<i>Tetrastichus giffardianus</i>	
1915.....	28,010	31.5	0.3	5.9	0.2	37.9
1916.....	83,304	17.2	13.3	2.1	.6	33.2
1917.....	72,139	12.7	20.3	7.3	7.2	47.5
1918.....	63,480	12.4	34.6	2.6	6.2	55.8
1919.....	75,406	9.4	19.6	1.6	7.6	38.2
1920.....	57,406	9.4	22.7	12.1	7.7	51.9
1921.....	88,616	7.6	26.9	5.5	16.4	56.4

Diachasma tryoni is shown in Table 5 to have been the most effective parasite since 1917, and was responsible for 47.7 per cent of the total parasitism in 1921. *Tetrastichus giffardianus* has been increasing slowly in value until in 1921 its effectiveness was more than double that of any previous year, and it had advanced to second place in importance. No particular reason for the sudden increase of this species in 1921 has been observed, but it was probably the result of favorable host and climatic conditions.

SUMMARY

The infestation of fruits in Hawaii by *Ceratitis capitata*, and the work of the four introduced species of parasites during 1921, have not been to any great extent different from what they were in the four previous years. There are, however, a few notable points which have been brought out in these records.

The average infestation per fruit in 1921 was higher than usual in a number of host fruits, but this condition was accompanied by the highest yearly parasitism on record.

Percentage of parasitism by *Opius humilis* continues to decrease, and this parasite is still in third place, from the standpoint of parasitism over yearly periods; and *Tetrastichus giffardianus* has advanced from fourth to second place.

The average yearly parasitism by all four parasites for the 5-year period 1917-1921 has been approximately 50 per cent. This destruction of one-half of all *Ceratitis capitata* about Honolulu has kept down the number of adults, so that the less preferred host fruits of commercial value are harvested with little or no infestation, if removed from the trees as early as possible without impairing their value.

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A HOMOGENEOUS CARBON DISULPHIDE EMULSION¹

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INTRODUCTION

Carbon disulphide, emulsified in water by means of soaps, is now being used throughout the area infested by the Japanese beetle, *Popillia japonica* Newm., to destroy the immature stages of this insect in the soil. All of these concentrated carbon disulphide-water emulsions tend to stratify into two or more layers of different composition which must be agitated to form homogeneous mixtures.

If a portion of such a concentrated emulsion were diluted without agitation and applied to the soil about the roots of plants, the dilution made from the heavier layer would have an excessive proportion of the insecticide and would be likely to injure the plants, while the dilution made from the lighter layer might not be strong enough to kill the insect. Experience has shown that these concentrated carbon disulphide-water emulsions were not entirely satisfactory, owing to the variability in the concentration of the dispersed phase, and experiments were conducted to make an emulsion of carbon disulphide which would not have this undesirable property.

EXPERIMENTAL WORK

As all of these emulsions contain two immiscible liquids—carbon disulphide and water—it was thought that a more satisfactory concentrated emulsion could be made by substituting for the water some other liquid which is more miscible with carbon disulphide. Very good concentrated mixtures of this type were made by dissolving the carbon disulphide in methyl or ethyl alcohol. The dilute emulsions were made by precipitating the carbon disulphide from the alcoholic solution by the addition of water. This procedure, however, which was used in the case of certain oils by Lewis (6) and by Joshi (5), is effective only when there is a very small proportion of carbon disulphide in the alcoholic mixture. Since it is desirable to have a large proportion of carbon disulphide in these mixtures, it was thought necessary to add a suitable third substance.

Investigators have observed that the addition of 5 per cent methyl alcohol to mixtures of ethyl alcohol and petroleum (1) increased the stability of the mixtures when diluted with water. Transparent homogeneous emulsions can be made by adding glycerol to a solution of calcium oleate in carbon tetrachloride (2, p. 30). A solution of a cresol soap (4) has been prepared from linseed oil, potassium hydroxide, alcohol, water, and crude cresol, and a liquid soap with formaldehyde (3) has been prepared from castor oil saponified with alcoholic

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potassium hydroxide, water, and formaldehyde; but, so far as the writer has been able to determine, no concentrated mixture of carbon disulphide has been prepared with alcoholic potassium hydroxide and oleic acid.

The preliminary experiments for determining the nature of the third substance to be added to the carbon disulphide-alcohol mixture indicated that a soap is the best addition for increasing its stability with water. The sodium soap formed from oleic acid and different proportions of sodium hydroxide were not sufficiently miscible with carbon disulphide to be satisfactory, but the potassium soaps formed in the same manner mixed well with carbon disulphide and alcohol.

The next step was to determine the maximum volume of carbon disulphide and the optimum quantities of alcohol, oleic acid, and potassium hydroxide to be used. Alcoholic potassium hydroxide² was mixed with U. S. P. oleic and 95 per cent ethyl alcohol, in the proportions of hydroxide 42 c. c., oleic acid 48.5 c. c., and ethyl alcohol 8 c. c. This mixture contained about 2 per cent excess of oleic acid over the quantity required to react with the potassium hydroxide to assure complete conversion of the potassium to potassium oleate, and thus prevent the loss of carbon disulphide by the action of potassium alcoholate, converting it to potassium xanthate. This alcoholic potassium oleate was mixed with carbon disulphide in the proportions of 1:9, 2:8, 3:7, 4:6, and 5:5, by volume. Although the lower proportions of the alcoholic potassium oleate gave concentrated mixtures having the best appearance, there was not sufficient soap to disperse the carbon disulphide in water. The mixture 2:8 seemed to have the minimum proportion of soap necessary to keep the mixture from disintegrating when diluted with water.

The results having indicated that 70 per cent was near the maximum proportion of carbon disulphide which should be put into the mixture, the percentage of excess ethyl alcohol was varied from 0 to 22, and the corresponding percentage of alcoholic potassium oleate from 30 to 8. Each of these 70 per cent carbon disulphide emulsions was diluted by pouring 5 c. c. of water into 5 c. c. of the emulsion, and, after slight agitation, adding it to a large volume of water. It was not possible to produce a homogeneous dilution by pouring any of these emulsions directly into a large volume of water, but it was possible to obtain such dilutions after the initial dilution obtained by adding an equal volume of water to the concentrated emulsion. These mixtures made good dilutions with water, the stability of the dilution being proportionate to the amount of soap present. The results of these different mixtures, which are outlined in Table 1, indicate that the mixture containing 14 per cent ethyl alcohol, 8.56 per cent oleic acid, and 7.44 per cent potassium alcoholate was the best. Expressed in other terms, the composition of a liter of the best alcoholic carbon disulphide emulsion is:

C. P. carbon disulphide.....	700.00 c. c.
95 per cent ethyl alcohol.....	214.40 c. c.
U. S. P. oleic acid.....	85.60 c. c.
U. S. P. potassium hydroxide.....	14.88 gm.

² Alcoholic potassium hydroxide (potassium alcoholate) was prepared by refluxing 100 gm. of U. S. P. potassium hydroxide with 400 c. c. of 95 per cent ethyl alcohol, and, after cooling, filtering the supernatant liquid through a dry filter. The alcoholate was standardized by titrating a diluted aliquot part against 0.1N hydrochloric acid, using methyl orange as an indicator, and so adjusted with 95 per cent ethyl alcohol as to contain 0.2 gm. of potassium hydroxide per centimeter.

TABLE 1.—*Characteristics of insecticidal emulsions and their stability when diluted; composed of 70 per cent of carbon disulphide and various proportions of alcohol, oleic acid, and potassium alcoholate*

Proportions of—			Characteristics of concentrate	Stability of dilution, 5 : 100 of water
Ethyl alcohol (95 per cent)	Oleic acid	Potassium alcoholate		
<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		
0	16.05	13.95	Considerable flocculent precipitate.....	Very good.
2	14.98	13.02do.....	Very good.
4	13.91	12.09	Flocculent precipitate.....	Very good.
6	12.84	11.16do.....	Very good.
8	11.77	10.23do.....	Good.
10	10.70	9.30	Slight flocculent precipitate.....	Good.
12	9.63	8.37	Transparent amber solution.....	Good.
14	8.56	7.44do.....	Good.
16	7.49	6.51do.....	Fair.
18	6.42	5.58do.....	Fair.
20	5.35	4.65do.....	Fair.
22	4.28	3.72do.....	Poor.

ADDITION OF OILS OR FATTY ACIDS

Further experiments were made to determine whether the dispersion of the carbon disulphide in water could be facilitated by the addition of oils or fatty acids to the concentrated emulsion. Mixtures were prepared containing 70 per cent carbon disulphide and different proportions of alcoholic potassium oleate (95 per cent ethyl alcohol, 214 c. c.; U. S. P. oleic acid, 86 c. c.; and U. S. P. potassium hydroxide, 15 gm.), together with coconut oil, cottonseed oil, copaiba oil, linseed oil, olive oil, oleic acid, or lactic acid. It was found that satisfactory concentrated mixtures could be made with coconut oil, cottonseed oil, linseed oil, and olive oil. Of these, the mixture in which 10 per cent of the alcoholic potassium oleate was replaced by cottonseed oil appeared to be the most efficacious in facilitating the dispersion of the carbon disulphide in water. The formula of the carbon disulphide emulsion was therefore modified to be as follows:

C. P. carbon disulphide.....	700 c. c.
U. S. P. oleic acid.....	77 c. c.
Ethyl alcohol (95 per cent).....	193 c. c.
Cottonseed oil (purified).....	30 c. c.
U. S. P. potassium hydroxide.....	13.5 gm.

To prepare a liter of this alcoholic emulsion, dissolve an excess of potassium hydroxide in alcohol; then filter off the insoluble carbonate and, after determining the hydroxide content by titration against a standard acid, add sufficient alcohol to obtain a concentration of 13.5 gm. of potassium hydroxide in 193 c. c. of alcohol; then pour 77 c. c. of oleic acid into each 193 c. c. of the alcoholic potassium hydroxide solution; then add 700 c. c. of carbon disulphide and 30 c. c. of cottonseed oil to each 270 c. c. of the oleic acid-alcoholic solution.

The resulting emulsion is amber colored, transparent, and homogeneous over a relatively long period. It is readily measured in small quantities, and mixes well with water. It must be diluted initially with an equal volume of water before being mixed with the larger quantity of water used in the insecticidal treatment in order to obtain a good dispersion of the carbon disulphide.

SUMMARY

A concentrated carbon disulphide emulsion has been prepared for insecticidal use which is transparent, homogeneous, and readily measured in small quantities. It is necessary to pour an equal volume of water into the emulsion and agitate it before adding it to the larger volume of water used in the insecticidal treatment. The composition of this emulsion is given.

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CARDIOCHILES NIGRICEPS VIER., AN IMPORTANT PARASITE OF THE TOBACCO BUD WORM, *HELIOTHIS VIRESCENS* FAB.¹

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INTRODUCTION

The most effective insect enemy of the tobacco bud worm, *Heliothis virescens* Fab., in the southern tobacco region² of the United States is the ichneumon fly, *Cardiochiles nigriceps* Vier.

While studying the biology and control of various tobacco insect pests at Quincy, Fla., the writers made observations and notes on the life history and habits of this parasite. The present paper summarizes the data collected.

HISTORY

Cardiochiles nigriceps was first described in 1912, by Viereck.³ In 1917 Morgan and McDonough⁴ made brief mention of the economic importance of this bud-worm parasite under the name *Toxoneura* sp. Aside from these two references, no mention has been found in the literature regarding this insect.

ECONOMIC IMPORTANCE

From the standpoint of economic importance, *Cardiochiles nigriceps* stands easily first among several species of parasites that attack the tobacco bud worm in the tobacco-growing sections of Georgia and Florida. Several seasons' observations would indicate that it remains fairly constant in abundance from year to year.

The economic value of this parasite is very difficult to determine, owing to the high mortality of young bud worms under laboratory conditions. It appears, however, that many young bud-worm larvae die very quickly as the result of its attack, although the majority approach maturity before they succumb. As the tobacco bud-worm larva continues to do serious damage to tobacco buds throughout its entire period of growth, it is evident that the benefit derived from *Cardiochiles nigriceps* is more cumulative than immediate.

During the early part of the tobacco-growing season the parasite is observed only rarely, but as the season advances its numbers rapidly increase. In July and August parasitism by this species frequently averages from 50 to nearly 100 per cent.

¹ Received for publication Dec. 15, 1925; issued July, 1926.

² Northern Florida and southern Georgia.

³ VIERECK, H. L. DESCRIPTIONS OF ONE NEW FAMILY, EIGHT NEW GENERA, AND THIRTY-THREE NEW SPECIES OF ICHEUMON-FLIES. U. S. Nat. Mus. Proc. 43: 575-593. 1912.

⁴ MORGAN, A. C., and McDONOUGH, F. L. THE TOBACCO BUD WORM AND ITS CONTROL IN THE SOUTHERN TOBACCO DISTRICTS. U. S. Dept. Agr. Farmers' Bul. 819, 12 p., illus. 1917.

All attempts to breed *Cardiochiles nigriceps* from hosts other than *Heliothis virescens* have yielded negative results; nor has it been reported as parasitic on other insects. It appears, however, to attack the bud worm on its various food plants other than tobacco. The percentage of parasitism on beggarweed (*Meibomia* sp.), which is the favored food plant of the tobacco bud worm during the early fall months, is usually much less than on tobacco at the same season. This is apparently due to the fact that beggarweed is scattered over

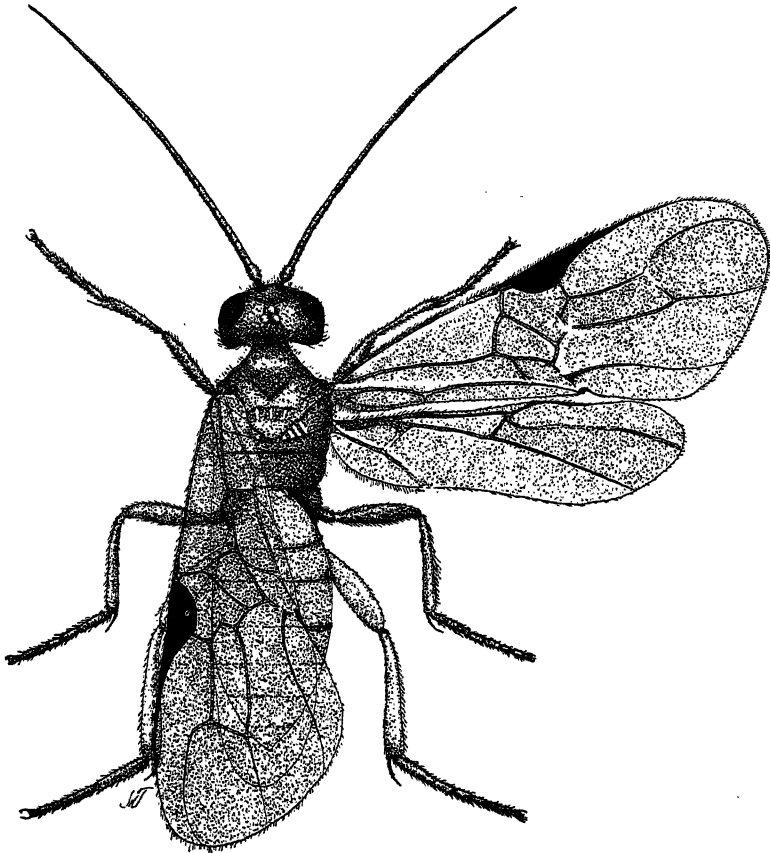


FIG. 1.—Adult of *Cardiochiles nigriceps*

great areas of land, whereas tobacco is concentrated on relatively small areas. If the last brood of bud worms in the fall confined its attack to tobacco suckers, the benefit derived from *Cardiochiles nigriceps* would doubtless be much greater.

DISTRIBUTION

It appears that *Cardiochiles nigriceps* is limited to the southeastern part of the United States. Many observations show that it is abundant in the Georgia and Florida tobacco-growing sections. It has also been reported from Alabama and Mississippi, and has been found as far north as Huntsville, Ala., and Clarksville, Tenn.

CLASSIFICATION AND DESCRIPTION

Cardiochiles nigriceps is a member of the hymenopterous superfamily Ichneumonoidea and family Braconidae. The adult (fig. 1) has been well described by Viereck,⁵ as follows:

Type-locality.—Savannah, Georgia.

Type.—Cat. No. 15007, U.S.N.M.

Cardiochiles nigriceps, new species. *Female*.—Length 7.4 mm.; head including antennae and palpi, prescutum, scapulae, pleurae, wings, coxae, trochanters, fore and mid femora, hind tarsi, tips of hind tibiae and propodeum mostly black or blackish, elsewhere, excepting fore and mid tibiae and tarsi which are dark brown or blackish, mostly reddish. Related to *C. viator* (Say) and *C. seminigra* (Cresson).

Allotype.—Essentially as in the type.

Labeled, "*Toxoneura nigriceps* Riley. No. 3956,^o reared Aug. 10, 1886, from *Chloridea rhexia*, received from Mr. R. S. Barnoll."

A series of female paratypes labeled "Am. Sumatra Tobacco Co.," from Quincy, Florida, shows variation in color, one specimen in particular having the prescutum and scapulae almost entirely reddish and the mid femora with a reddish stain. These departures from the typical specimens would seem to indicate that this species may prove to be a race of *C. viator* (Say) or *C. seminigra* (Cresson).

THE EGG

The egg (fig. 2) just before deposition, is slender, elongate, sharply pointed at one end and more bluntly pointed at the other; often slightly curved, decidedly thickened in the middle, and translucent white. The average length is about 0.57 mm. and the average greatest diameter is 0.11 mm.

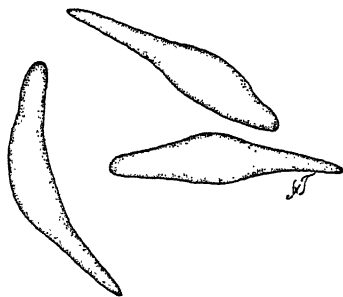


FIG. 2.—Eggs of *Cardiochiles nigriceps*

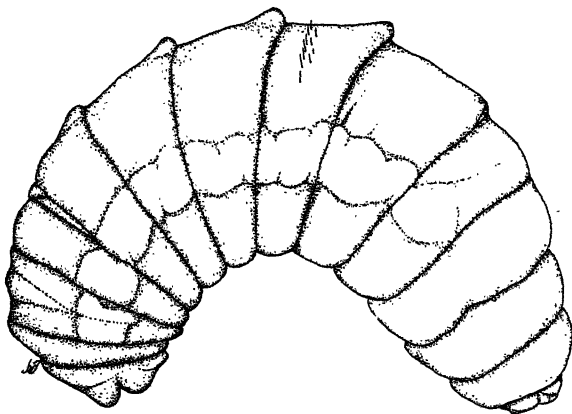


FIG. 3.—Larva of *Cardiochiles nigriceps*, lateral view

THE LARVA

The mature larva (fig. 3), as it emerges from the host, is pale yellowish green. When viewed dorsally it appears elliptical, slightly broader posteriorly. Viewed laterally, it appears more or less cres-

⁵ VIERECK, H. L. Op. cit.

cent shaped, with a distinct, submedian dorso-ventral muscular ridge extending over all but a few of the segments at caudal and cephalic extremities. The well-defined body segments are 13 in number. The head is almost entirely retracted and is not chitinized; the mandibles are small and practically invisible. The average dimensions are approximately 9.4 mm. in length, when extended, and 3.4 mm. in depth, dorso-ventrally.

THE PUPA

The pupa (fig. 4) at first is creamy white in color, and resembles the adult in shape. The appendages are closely folded about the body. The pupa gradually darkens, until the characteristic adult colors appear and can be dimly seen through the silken-walled cocoon.

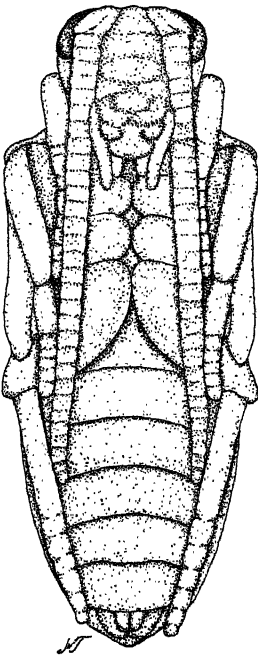


FIG. 4.—Pupa of *Cardiochiles nigriceps*, ventral view

LIFE HISTORY

THE EGG PERIOD

All attempts to dissect the egg of *Cardiochiles nigriceps* from the body of its tiny host, or to ascertain the exact length of the incubation period, have been unsuccessful.

The period of oviposition usually begins about the middle of April, when the young tobacco plants are just becoming established in the fields, and continues until about the middle of September, when young bud worms are becoming scarce.

THE LARVAL PERIOD

As it proved impossible to ascertain the time when the egg hatched, the beginning of the larval period could not be determined. The combined incubation and larval periods (including the prepupa), however, ranged from about 17 to 21 days in midsummer.

The larva feeds within the body of its host, developing as the bud worm develops, until maturity is reached. The host seldom succeeds in growing to normal size, but usually enters the soil when apparently only about two-thirds grown. The parasitized bud worm never succeeds in pupating. When fully mature the parasite eats a small hole through the dorsal or lateral surface of its host, and slowly emerges. Nothing but the empty skin of the bud worm is left. All records indicate that only one parasite can complete its development in each host larva.

Shortly after emergence, the larva begins to spin a cocoon. This cocoon is made by the larva turning around and around in the soil, forming a cell and spinning a tight silken wall. The cocoon can be formed only underground. When the larva is placed on top of the earth it produces a mass of silk, but is unable to complete a cocoon.

THE COCOON

The cocoon is broadly ellipsoidal in shape, dirty white, and very tough. In general aspect it rather closely resembles the cocoons of some of the larger Lepidoptera. In size it is usually 8 to 9 mm. long and 3 to 4 mm. in diameter.

THE PREPUPAL PERIOD

After completion of the cocoon there is an interval of approximately four days before pupation takes place. This interval must be considered as a prepupal stage, in which the larva is gradually contracting and preparing to pupate.

THE PUPAL PERIOD

It has been determined by repeated dissection of cocoons that pupation takes place on or about the fourth day after the cocoon is formed. During the summer the length of the pupal period usually ranges from 8 to 12 days.

THE ADULT PERIOD

Just how long the adult insect lives under normal conditions seems never to have been established. In the laboratory very few were kept alive more than four or five days. When caged, they strive continually to escape, and all efforts to induce feeding, copulation, or oviposition failed.

HABITS

OVIPOSITION

When the weather is bright and warm, *Cardiophiles nigriceps* may be observed in tobacco fields at almost any hour of the day. Ovipositional activity is much greater in the middle of the day. In cool, cloudy weather the parasite appears to be sluggish, and seeks concealment and protection beneath tobacco leaves, weeds, or debris.

The female parasite prefers as its victims bud worms in the first instar, although oviposition frequently occurs in second-instar larvae. Only one instance has been observed where an egg was apparently deposited in a larva of the third instar. Large bud worms are ignored.

The adult flies slowly from plant to plant, hovering about each one for some little time. Frequently the search seems to be made quite systematically. The insect goes carefully over the entire upper portion of the plant, usually keeping about an inch from the leaf surface. It gives special attention to the buds and suckers. The tiny bud worm appears to be located by the senses of touch and smell. Often the parasite will alight and examine some particular spot more closely, feeling carefully with her antennae. Frequently, if there are signs of fresh eating on the leaf, she seems to become much excited. The ovipositor is extruded, and the insect makes tentative stabs as though trying to locate the missing bud worm. Often she will walk over a bud, exploring it carefully with her antennae and thrusting her ovipositor into every crack and fold in an attempt to reach the tiny larva in the center of the bud.

When a bud worm is located, the parasite evinces signs of intense excitement. She taps the tiny worm all over with her antennae, sometimes even knocking it away in her movements. When she has determined the exact position of the larva, she straddles it, and, extruding her ovipositor, attempts to thrust it into her victim. Sometimes she repeatedly misses it and has to turn apparently to relocate the worm. The ovipositor is thrust either into the dorsal or lateral surface of the larva, usually about the middle, but quite frequently near the caudal or the cephalic end. The act of oviposition requires only about two or three seconds. As soon as the ovipositor is withdrawn the parasite immediately begins searching for another victim.

There appears to be a marked tendency on the part of *Cardiochiles nigriceps* to avoid ovipositing in a bud worm which has previously been parasitized. A female will frequently return to a tobacco plant which she has left only a short time before, but she ignores the larvae which she parasitized on her former visit. Very often a female will refuse to oviposit in young bud worms of the preferred size, and it is inferred that they have previously been parasitized.

For two or three minutes after an egg is deposited, the bud worm appears to be completely paralyzed. When it is noted that the inserted egg is often from one-third to one-half the length of the larva, this is not surprising. In a short time, however, the bud worm begins feeding again.

OTHER BEHAVIOR

As already mentioned, the present writers have no knowledge of *Cardiochiles nigriceps* ever having been bred from any insect other than *Heliothis virescens*. In two instances, however, this parasite appeared to attempt oviposition in first-instar tobacco hornworms (*Protoparce sexta* Joh.). In each instance the hornworms were reared to the pupal stage, but no signs of parasitism developed. It is very unlikely that the hornworm can serve as a host for this parasite.

Numerous adults of *Cardiochiles nigriceps* have been collected in tobacco fields, and examinations have shown that the sexes are about equally represented, but the writers have never observed copulation.

Although the adult insect manifests great excitement under cage conditions, in the field it shows no fear of man. A female will work undisturbed with an observer standing close beside the tobacco plant. The stalk upon which she is resting may even be bent downward for closer observation without disturbing her.

SEASONAL HISTORY

In the vicinity of Quincy, Fla., adults of *Cardiochiles nigriceps* appear in the spring soon after tobacco has been set in the fields, usually from the first to the middle of April. By May they are usually observed in considerable numbers, and from then until the middle of September they are very abundant. After the middle of September the numbers decline, although specimens have been observed in the field as late as November 3. Emergence of adults in outdoor cages has been recorded from March 20 until September 11.

There are probably three or four generations a year in this locality, but the last two are much the largest. In July and August this parasite is so abundant in tobacco fields that frequently three or four may be observed flying about a single stalk of tobacco. At this season of the year 50 to nearly 100 per cent parasitism by this species is not unusual.

Cage records checked with field observations indicate that the winter is passed in the pupal stage, in the soil below the surface.

SUMMARY

Cardiochiles nigriceps is the most important insect parasite of the tobacco bud worm, *Heliothis virescens*, in the southeastern part of the United States. In July and August parasitism by this species frequently ranges from 50 to nearly 100 per cent.

As far as the writers are aware, this parasite attacks only the true bud worm of tobacco, *Heliothis virescens*.

At Quincy, Fla., the combined incubation and larval periods required from 17 to 21 days during the summer months. The pupal period ranged from 8 to 12 days.

VITAMIN A IN POULTRY FLESH AND FAT¹

By RALPH HOAGLAND, *Biochemist, Biochemic Division*, and ALFRED R. LEE, *Associate Poultry Husbandman, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In a previous investigation² the writers studied the vitamin B content of poultry flesh and eggs. In this paper they report the results of experiments conducted with rats for the purpose of determining the vitamin A value of the body flesh and fat of several species of domestic fowl. These tests were made on fowls reared under ordinary conditions and fed rations which were in general use. The vitamin A content of eggs has already been reported by several investigators, but there appears to be no information in the literature concerning the amount of vitamin A in the products tested by the writers.

EXPERIMENTAL WORK

FEEDING HISTORY OF FOWLS TESTED

Since it is probable that the vitamin A content of the flesh and fat of poultry is directly related to the amount of that vitamin in the diet of the birds, it seems desirable to record briefly the feeding history of each lot of fowls tested.

Barred Plymouth Rock hens.—This lot of birds consisted of four hens which had been raised on the experiment farm of the United States Department of Agriculture at Beltsville, Md. These chickens, which were hatched in the spring of 1923, had free grass range during the growing period, and were fed a ration previously described by the writers³ until 6 months old. The pullets were then put in laying pens with moderate-sized yards which contained a fair amount of grass during the growing season. The ration fed to the hens from the time they were 6 months old until October, 1924, consisted of scratch feed made up of equal parts of corn, wheat, and oats, and a mash containing 4 parts rolled oats 2.5 parts meat scrap, 2 parts bran, 2 parts corn meal, and 1 part middlings, to which was added 5 per cent ground bone and 1 per cent salt. In addition, during the fall and winter months the hens were given a light feed of sprouted oats or cabbage two or three times a week. From October 1 to December 15, 1924, the hens received only scratch feed, after which they were fed mash also, until killed January 13, 1925, when the average live weight of the hens was 5.5 pounds.

Rhode Island Red hens.—This lot of birds consisted of five hens, 22 months old, which had also been raised on the experiment farm at Beltsville. These hens were raised and kept under practically the same conditions as those described for the Barred Plymouth Rock hens. The average live weight of the hens when killed, January 31, 1925, was 5.5 pounds.

Muscovy ducks.—These ducks—three small females and one large male—were purchased from a farm in Beltsville. The male bird was hatched in 1923 and the females in 1924. They were fed lightly on a commercial laying mash supplemented with corn meal and whole corn, and they had free range on which there was an abundance of green feed. No additional green feed was provided during the winter months, but the ducks were able to get considerable green stuff from the range during the early part of that season. The ducks were killed February 25, 1925, the average live weight at the time being 6.3 pounds.

¹ Received for publication Nov. 19, 1925; issued July, 1926.

² HOAGLAND, R., and LEE, A. R. ANTINEURITIC VITAMIN IN POULTRY FLESH AND EGGS. *Jour. Agr. Research* 28: 461-472, 1924.

³ HOAGLAND, R., and LEE, A. R. *Op. cit.* p. 463.

Pekin ducks.—These birds—3 females and 1 male—were obtained from a dealer who had purchased them from a farmer in Virginia. The ducks were hatched in the spring of 1924, but no information is available concerning their feeding on the farm. The dealer held these ducks for about a month before slaughter, during which time they received only a limited amount of green feed. The ducks were slaughtered March 7, 1925, the average live weight at the time being 4.75 pounds.

Toulouse geese.—Two female geese, approximately 2 years old, were purchased from a farmer near Beltsville. They were raised on free range, and received corn as grain. During the winter previous to slaughter they had a rye pasture and were fed a commercial dairy mash mixed with skim milk. The geese were in good flesh when slaughtered, January 15, 1925, the average live weight being 12.2 pounds.

Emden geese.—Three ganders were purchased alive on the Washington, D. C., wholesale market February 28, 1925. They had probably been raised on near-by Virginia or Maryland farms, but no information concerning their feeding history was available. The geese were killed March 2, 1925, the average live weight at the time being 9.6 pounds.

White Holland turkeys.—One male bird, approximately 21 months old, was purchased February 2, 1925, from a farm near Beltsville. The turkey had been fed a commercial scratch grain, but no mash, and had been allowed free range on which there was a fairly good amount of green feed. The live weight of the bird at the time of slaughter was 23.4 pounds.

Mammoth bronze turkeys.—Two male birds, approximately 10 months old, were purchased from a dealer in poultry who conducts a small farm near Washington, D. C. He purchased the turkeys from farmers in Virginia about November 15, 1924, and held them on his farm for about three months until they were purchased for this experiment. During this period they were allowed a very moderate range, but the amount of green feed was limited at that season of the year. The turkeys were fed only corn during this time. No information is available concerning the conditions under which the turkeys were raised on the farm. The average live weight of the turkeys when killed February 11, 1925, was 15.5 pounds.

Pearl gray guinea fowls, lot 1.—Eight young female guinea fowls, hatched in the spring of 1924, were purchased from a farmer near Hyattsville, Md. The young guinea chickens were started on a commercial chick feed and were continued on it for a few weeks, and then they were allowed free range. After that they were fed a small amount of corn and wheat, but received no other feed except what they picked up. The amount of green feed on the range during the winter months was undoubtedly very limited, but it was probably greater than that available for the Plymouth Rock and Rhode Island Reds hens from the experiment farm at Beltsville. The average live weight of the guinea hens at the time of slaughter, January 23, 1925, was 2.7 pounds.

Pearl gray guinea fowls, lot 2.—Seven male and two female guinea fowls were purchased from a dealer who had bought them from a farmer in Virginia. The dealer kept the birds on his farm for about a month after purchase, feeding them corn and allowing them free range, but the amount of green feed was limited at that time. No information was available concerning the feeding of the guinea fowls. The average weight of the birds at the time of slaughter, March 9, 1925, was 3.1 pounds.

Promptly after slaughter the muscle and fatty tissues were trimmed from the carcasses of each lot of fowls. The muscle tissue was ground, mixed with water and toluol, and dried to air dryness in a current of air at a temperature not higher than 60° C., the operation requiring less than 24 hours. The dried material was ground, transferred to glass bottles, and stored at a temperature approximating 4° C. until tested for vitamin A. The fatty tissues were rendered on a steam bath at a temperature not higher than 75° C., the operation requiring less than one hour. The rendered fat was filtered into glass jars, which were placed in cold storage at the temperature noted above. The samples of dried poultry flesh were analyzed for protein and fat and each sample of flesh and fat was tested promptly for vitamin A.

METHODS EMPLOYED IN TESTING FOR VITAMIN A⁴

The relative amounts of vitamin A in the samples of poultry flesh and fat were determined by feeding tests with young albino rats, the sample under examination constituting the only known source of this vitamin in an otherwise adequate ration. The rats were raised in the animal laboratory of the Bureau of Animal Industry. Litters containing more than 8 rats were reduced to that number on the day of birth, and those consisting of fewer than 6 were discarded. Only young rats which reached a weight of 40 grams within 30 days from birth were used for test purposes. The breeding rats were fed a ration relatively low in vitamin A.

Each sample of flesh, or fat, was mixed in one or more proportions in rations made up according to the following standard:

<i>Basal ration</i>		Parts
Protein ($N \times 6.25$)	-----	20
Ash	-----	4
Yeast	-----	10
Fat	-----	20
Starch to make	-----	100

The protein consisted of finely ground commercial casein which had been heated 72 hours in a current of air at an average temperature of 110° to 115° C. to destroy vitamin A. The ash mixture was made up according to a formula by Drummond and Watson.⁵ Dried bakers' yeast, hardened cottonseed oil, and cassava starch comprised the remainder of the ration. Casein was the only constituent of the basal ration found to require purification from vitamin A.

Each ration was fed to a group of 4 rats. Each rat was kept in an individual cage with a raised-screen bottom. The ration was supplied in a self-feeder, and a record was kept of the feed consumed. The rats were weighed biweekly and they were observed frequently for signs of ophthalmia.

FEEDING TESTS WITH BASAL RATIONS

Each ration containing poultry flesh or fat was fed to a group of rats from the start, without a preliminary test on a vitamin-A-free basal ration, as is the practice followed by many investigators. The procedure which the writers have followed in this work requires that proper allowance be made for the growth made by rats fed the basal rations alone under the same conditions as those existing in the other experiments.

In Figure 1 are shown the results obtained by feeding the basal ration to two groups of rats during this investigation. The rats made but limited growth, and all but two developed ophthalmia. The growth made by these rats probably is due largely, if not entirely, to the reserve store of vitamin A in their tissues, rather than to the presence of this vitamin in the basal ration.

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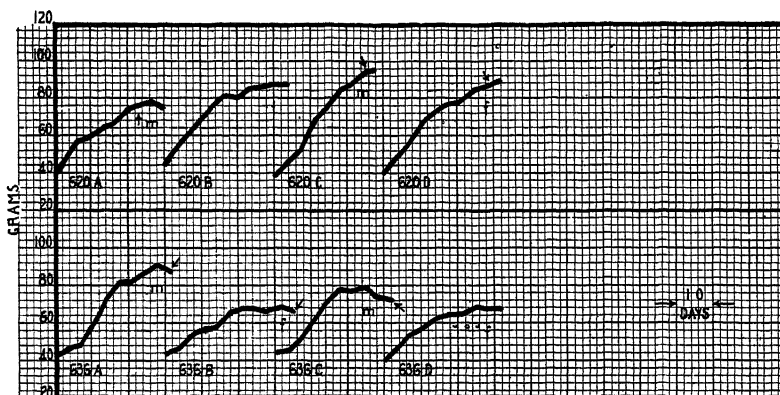


FIG. 1.—Growth of the rats which were fed the basal ration alone. Sex of rats in this and the other figures is indicated by the letters *m* and *f*; arrows indicate ophthalmia

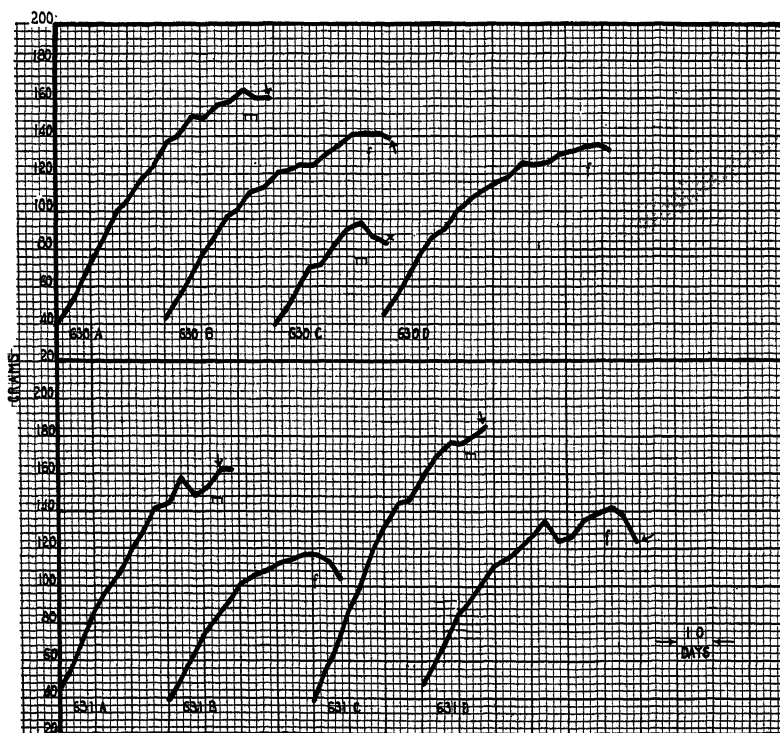


FIG. 2.—Growth of the rats fed the dried flesh of Barred Plymouth Rock hens as the source of vitamin A. The rats represented by the upper graphs were fed a ration containing 10 per cent of the flesh; and those by the lower graphs 20 per cent. Arrows indicate ophthalmia; *x* indicated severe rhinitis

VITAMIN A IN POULTRY FLESH

VITAMIN A IN CHICKEN FLESH

In Figure 2 are shown the growth curves of two groups of rats which were fed rations containing 10 and 20 per cent, respectively, dried Barred Plymouth Rock hen flesh. Rather irregular results were obtained, but by calculating the average gain in weight made by each group of rats it will be found that considerably better growth was made by those getting 20 per cent of the dried flesh. However, this proportion of the Plymouth Rock flesh did not furnish sufficient vitamin A for optimal growth.

In Figure 3 are shown the growth curves of two groups of rats which were fed rations containing 10 and 20 per cent, respectively, of dried

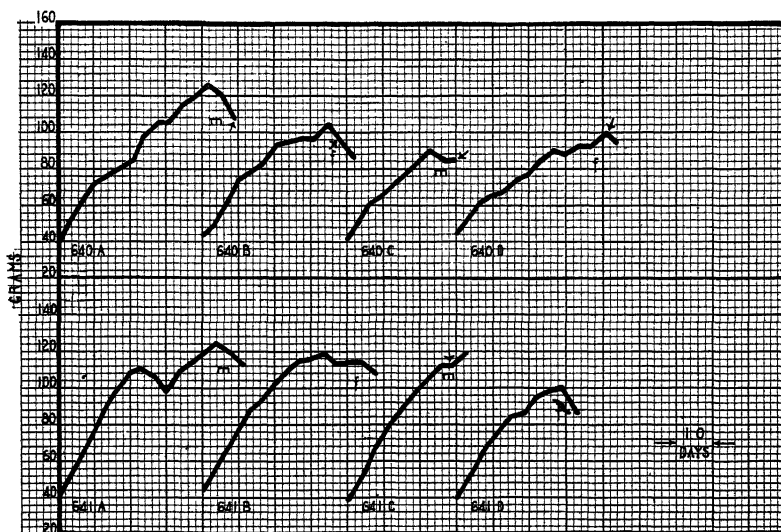


FIG. 3.—Growth of rats fed the dried flesh of Rhode Island Red hens as the source of vitamin A. Rats numbered 640 were fed a ration containing 10 per cent of the dried flesh. Those numbered 641 received a ration containing 20 per cent. Arrows indicate ophthalmia

Rhode Island Red hen flesh. The first group of rats (numbered 640) made very poor growth, and all developed ophthalmia. Although the second group of rats made somewhat better growth, it is apparent that 20 per cent of this lot of chicken flesh did not furnish nearly sufficient vitamin A for optimal growth.

On comparing Figures 2 and 3 it appears that the Barred Plymouth Rock hen flesh was appreciably richer in vitamin A than the Rhode Island Red hen flesh. Since both lots of birds were fed the same ration and handled in the same manner, it appears that the one lot of hens simply stored more vitamin A than did the other.

VITAMIN A IN DUCK FLESH

In Figure 4 are shown the results of feeding tests with the dried flesh from Muscovy and Pekin ducks, respectively. The first group of rats (numbered 657) was fed a ration containing 25 per cent of

Muscovy duck flesh, and the other group (numbered 660) received a ration containing 30 per cent of Pekin duck flesh. There is a remarkable difference in the amounts of vitamin A in these samples. All the rats getting the Muscovy duck flesh made excellent growth; in fact, rat No. 657A made extraordinary growth. On the other hand, the rats getting the Pekin duck flesh made very poor growth; three of them developed ophthalmia, and one died. It is to be noted, also,

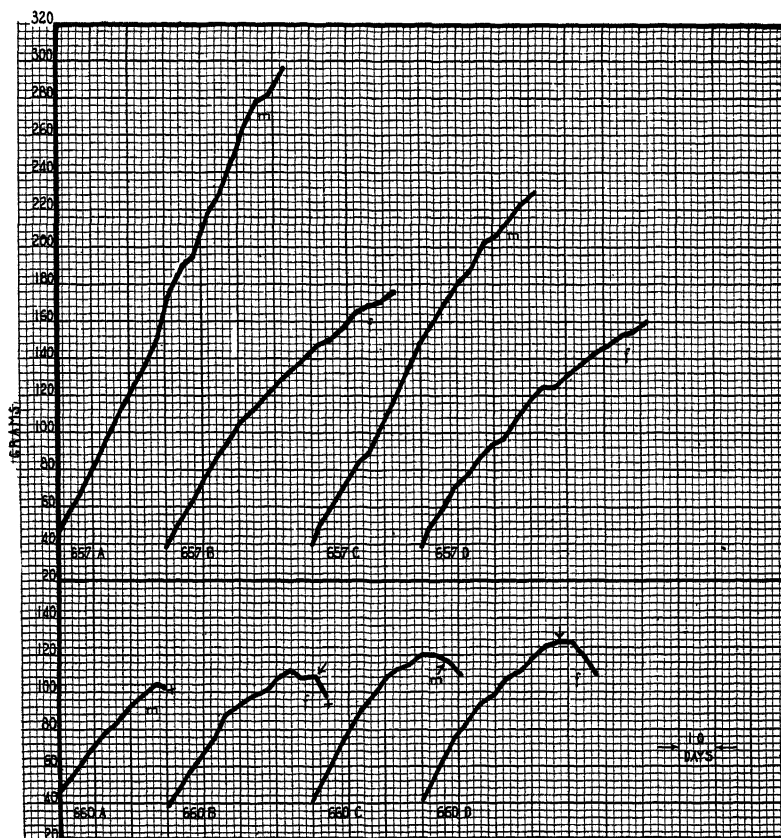


FIG. 4.—Growth of rats fed Muscovy and Pekin duck flesh as the source of vitamin A. Rats numbered 657 were fed a ration containing 25 per cent of dried Muscovy duck flesh. Those numbered 660 were fed a ration containing 30 per cent of dried flesh from Pekin ducks. Arrows indicate ophthalmia; crosses indicate death.

that there was 30 per cent of Pekin duck flesh in this ration as compared with 25 per cent of Muscovy duck flesh in the other ration.

The Muscovy ducks had received an abundance of green feed in addition to mash and grain, but the Pekin ducks had only a limited amount of green feed for a month before slaughter, and information on the history of their feeding is lacking.

VITAMIN A IN GOOSE FLESH

In Figure 5 are shown the results of feeding tests with the dried flesh from Toulouse and Embden geese, respectively. Rats numbered

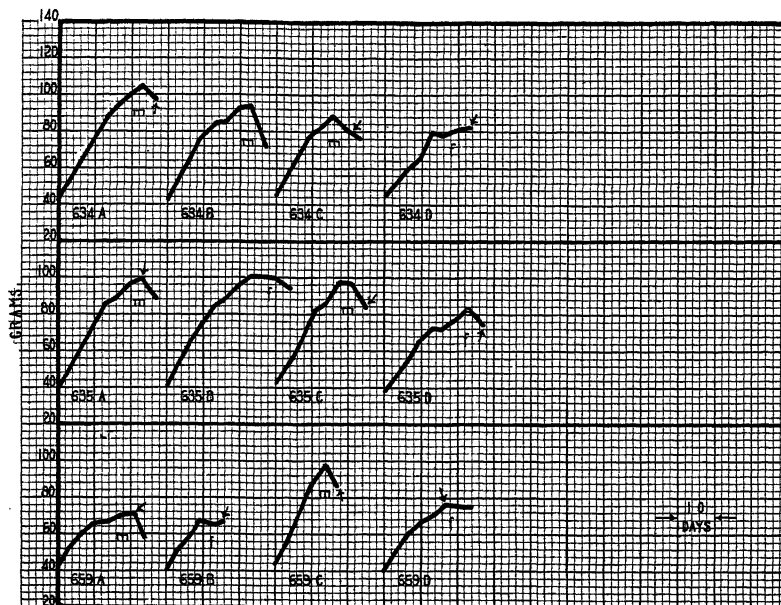


FIG. 5.—Growth of rats fed Toulouse and Embden goose flesh as the source of vitamin A. Rats numbered 634 and 635 were fed rations containing, respectively, 10 and 20 per cent of dried Toulouse flesh. Rats numbered 659 were fed a ration containing 30 per cent of Embden flesh. Arrows indicate ophthalmia

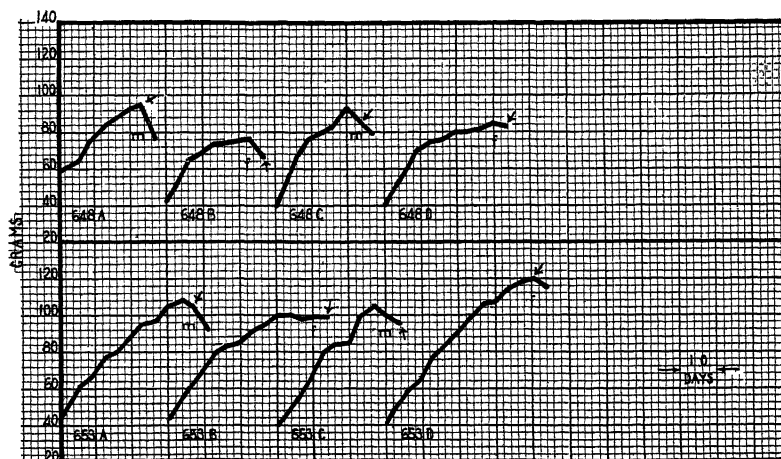


FIG. 6.—Growth of rats fed White Holland and Mammoth Bronze turkey flesh as the source of vitamin A. Rats numbered 648 were fed a ration containing 20 per cent of dried White Holland flesh. Those numbered 653 received a ration containing the same percentage of Mammoth Bronze flesh. Arrows indicate ophthalmia

634 and 635 were fed rations containing 10 and 20 per cent, respectively, of Toulouse goose flesh; and rats numbered 659 received a ration containing 30 per cent of Embden goose flesh. These graphs indicate that both lots of flesh were very poor in vitamin A, although that from the Toulouse geese appeared to contain slightly more than

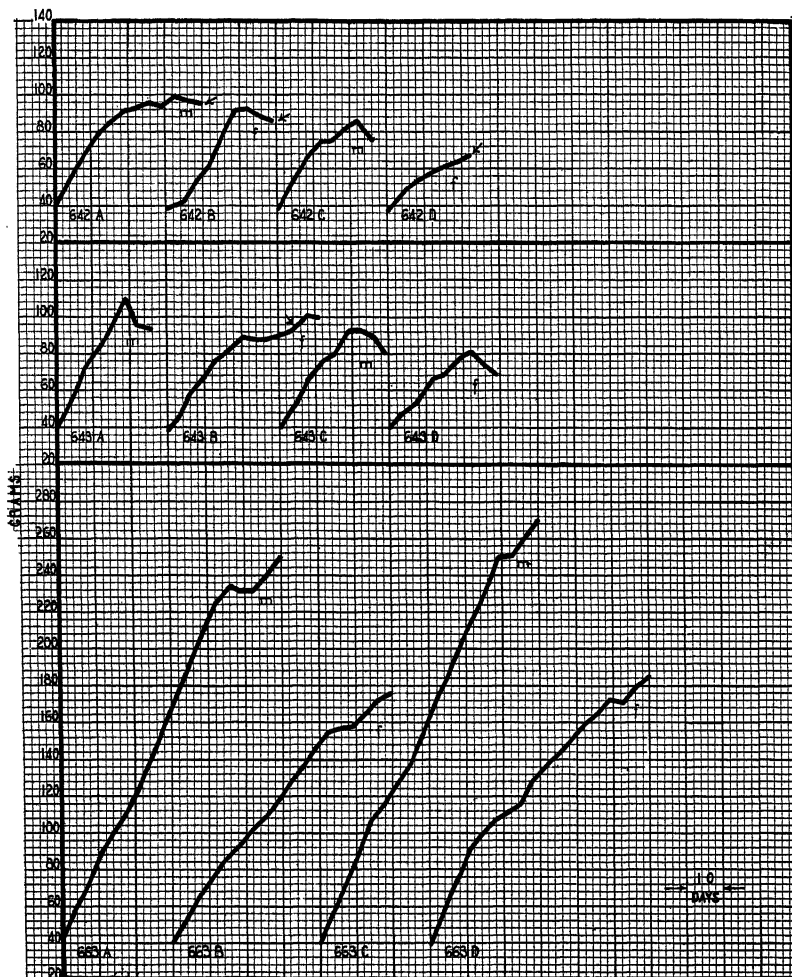


FIG. 7.—Growth of rats fed the dried flesh of guinea fowls as the source of vitamin A. Rats numbered 642 and 643 were fed rations containing, respectively, 10 and 20 per cent of flesh from the fowls purchased from a farmer near Beltsville, Md. Rats numbered 663 were fed a ration containing 30 per cent of flesh from fowls purchased from a dealer. Arrows indicate ophthalmia

than from the Embden geese. The Toulouse geese had been grown on free range with plenty of green feed, but the Embden geese were purchased from a dealer and no information concerning their feeding history was available.

VITAMIN A IN TURKEY FLESH

The results of the feeding tests with two samples of Turkey flesh are shown in Figure 6. Rats numbered 648 were fed a ration con-

taining 20 per cent of flesh from the White Holland turkey, and rats numbered 653 received an equal proportion of flesh from the Mammoth Bronze turkey. Apparently the latter sample of flesh contained slightly more vitamin A, but both samples were very poor in it.

VITAMIN A IN FLESH FROM GUINEA FOWLS

In Figure 7 are shown the results of feeding tests with the dried flesh from two lots of guinea fowls. Rats numbered 642 and 643 were fed rations containing 10 and 20 per cent, respectively, of the flesh

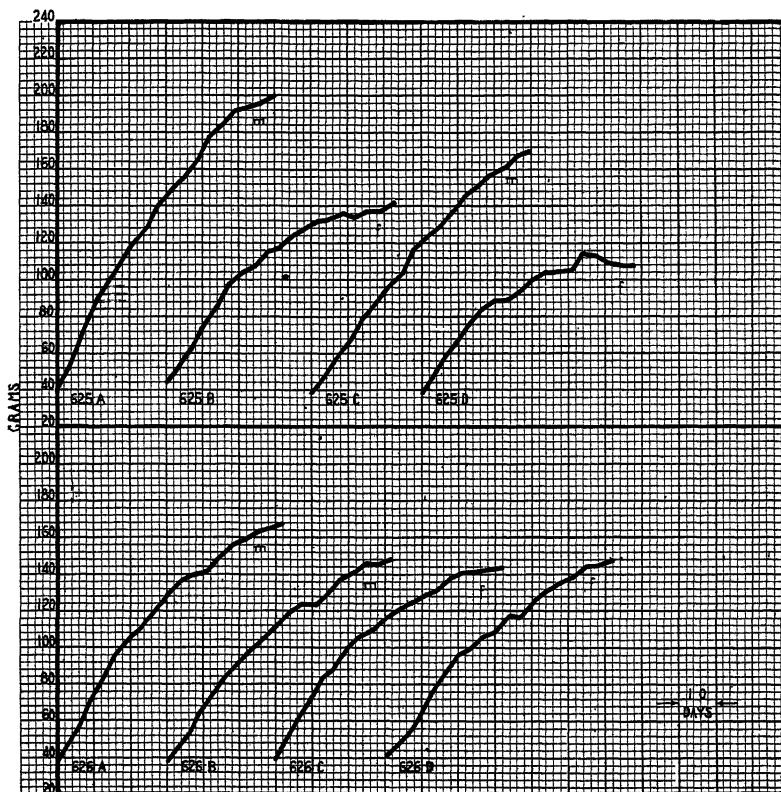


FIG. 8.—Growth of rats which received fat from Barred Plymouth Rock hens. The two groups of rats numbered 625 and 626 were fed rations containing, respectively, 5 and 10 per cent of the chicken fat

from the guinea fowls purchased from a farmer near Beltsville, and rats numbered 663 were fed a ration containing 30 per cent of the flesh from the other lot of guinea fowls which were purchased from a dealer. It is apparent from the growth curves of the rats that the first lot of guinea-fowl flesh was very poor in vitamin A. On the other hand all of the rats that were fed the second sample of flesh made remarkably good growth, and it is probable that a considerably smaller percentage of the flesh would have induced optimal growth. Although the greatest percentage that was fed of the first lot of guinea flesh was 20 per cent, whereas the second lot of flesh was fed to the

extent of 30 per cent of the ration, the difference in the growth made by the rats fed the two rations is so pronounced that it seems safe to conclude that the second sample of flesh was considerably richer in vitamin A than the first sample.

The guinea fowls purchased from a farmer near Beltsville had free range and plenty of green feed, but no information was available concerning the feeding of the other lot during the growing period. Apparently, however, the second lot of guinea fowls must have been fed a ration richer in vitamin A or had a much better range than the first lot, as indicated by the wide difference in the amounts of vitamin A in the two samples of flesh.

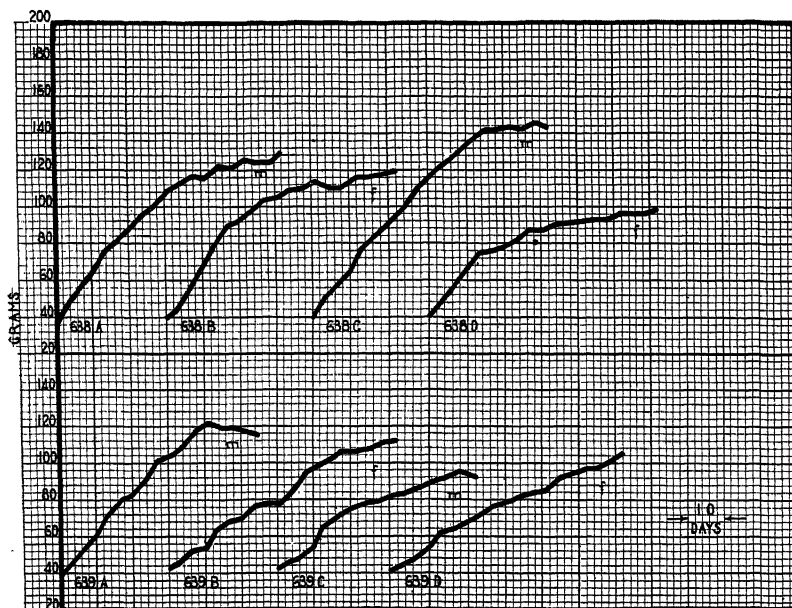


FIG. 9.—Growth of rats which received fat from Rhode Island Red hens as the source of vitamin A. Rats numbered 638 and 639 were fed rations containing, respectively, 5 and 10 per cent of the chicken fat

VITAMIN A IN POULTRY FAT

VITAMIN A IN CHICKEN FAT

The results of feeding test with rations containing fat from the Barred Plymouth Rock hens are shown in Figure 8. Although rats numbered 625 received only 5 per cent of the chicken fat, and rats numbered 626 received 10 per cent, yet the two groups made practically the same average gain in weight. Growth was considerably below normal.

In Figure 9 are shown the growth curves of two groups of rats fed rations containing 5 and 10 per cent, respectively, of fat from Rhode Island Red hens. Strangely, the rats receiving the larger proportion of chicken fat made the poorer growth. Comparing Figures 8 and 9, it seems that the fat from the Plymouth Rock hens was appreciably richer in vitamin A than that from the Rhode Island Red hens. A similar relation was previously noted concerning the amounts of

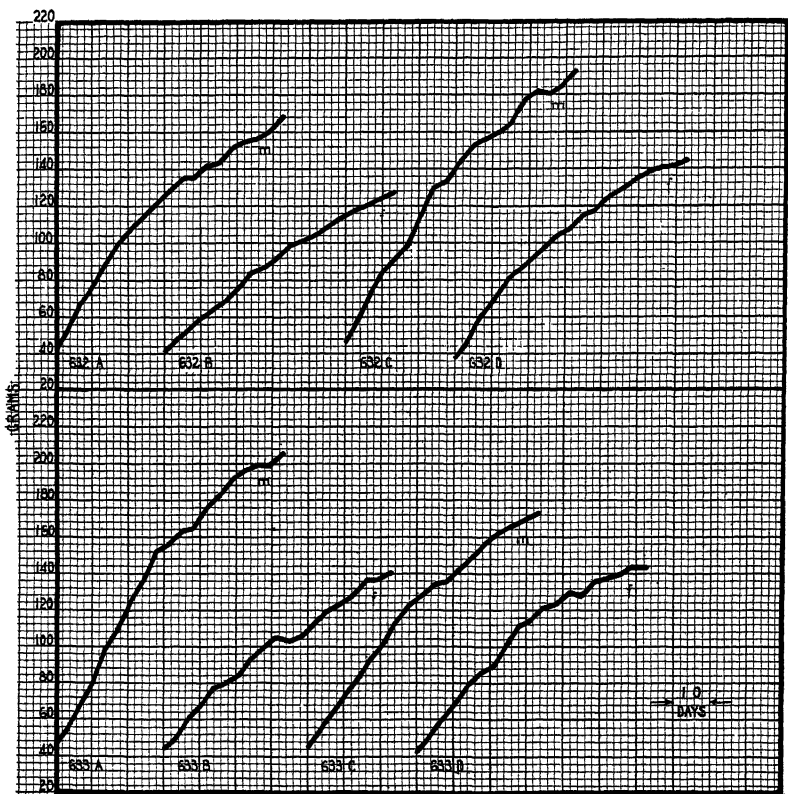


FIG. 10.—Growth of rats which received fat from Toulouse geese as the source of vitamin A. Rats numbered 632 and 633 were fed rations containing, respectively, 5 and 10 per cent of this fat

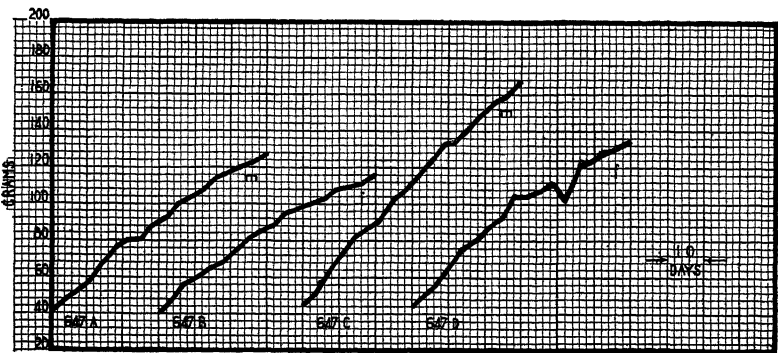


FIG. 11.—Growth of rats which received fat from White Holland turkeys. These rats were fed a ration containing 10 per cent of this fat

vitamin A in the flesh from the same two lots of hens. It is interesting to note that each lot of chicken fat was considerably richer in vitamin A than the corresponding lot of flesh (see figs. 2 and 3).

VITAMIN A IN GOOSE FAT

In Figure 10 are shown the results of feeding tests with rations containing 5 and 10 per cent, respectively, of fat from the Toulouse geese. Both groups of rats made fairly good, though not quite normal, growth, the rats getting 10 per cent of goose fat making a slightly greater average gain in weight. It appears that this sample of goose fat contained approximately the same amount of vitamin A as the fat from the Plymouth Rock hens (fig. 8). It seems, also, that the fat from the Toulouse geese was much richer in vitamin A than the flesh from the same birds (compare figs. 5 and 10).

VITAMIN A IN TURKEY FAT

In Figure 11 are shown the growth curves of rats fed a ration containing 10 per cent of fat from the White Holland turkey. Growth was considerably below normal. Comparing figures 11 and 6, it will be noted that the flesh from this turkey apparently contained much less vitamin A than did the fat from the same bird. This sample of turkey fat appeared to contain appreciably less vitamin A than the sample of goose fat (fig. 10).

SUMMARY OF RESULTS

In this paper are reported the results of a study of the concentration of vitamin A in the dried flesh from two lots each of chickens, ducks, geese, turkeys, and guinea fowls, and in the fat from two lots of chickens and one lot each of turkeys and geese.

The flesh from one lot each of ducks and of guinea fowls was found to be relatively rich in vitamin A; one sample of chicken flesh contained a fair amount of this vitamin; but the other samples of guinea fowl, duck and chicken flesh, and both samples of goose and turkey flesh, were relatively poor in vitamin A.

One sample of chicken fat and the sample of goose fat each contained a fair amount of vitamin A, but the sample of turkey fat was rather poor in this vitamin.

In each instance it was found that the sample of poultry fat was considerably richer in vitamin A than the corresponding sample of flesh.

On account of the limited number of samples of each kind of poultry flesh and fat which were tested for vitamin A, no general conclusions concerning the value of any one of these products as a source of this vitamin are justified. Additional information concerning the vitamin A content of poultry flesh and fat is to be desired.

BUD VARIATION AND CHIMERAS IN *MATTHIOLA INCANA* R. BR.¹

By HOWARD B. FROST

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INTRODUCTION

A mutant form of *Matthiola* described under the name Slender (4)² has been determined by Miss Mann (6) to be trisomic. Slender plants have 15 somatic chromosomes, while normals have 14. Genetic evidence (6) indicates that the extra chromosome of Slender is derived from the pair which carries the genes for single (normal) and double (petalomanous, sterile) flowers.

A case of bud variation has been described by the present writer (5),³ in which part of a plant was Slender single and the rest was normal double. This variation has been ascribed (6) to loss of one chromosome in early somatic development, changing a Slender single with one gene for singleness (Ddd) into a normal double (dd).⁴ The abnormal intermediate nature of the doubleness was ascribed to the formation of a periclinal single-double chimera as a result of the chromosomal change.

OBSERVATIONAL DATA

The present paper reports three cases of somatic variation and two of intermediate double flowers, all evidently to be explained in the same way as the bud variation previously described. Four of these variant plants occurred within a white, glabrous variety originally obtained under the name Snowflake (4), and the other plant came from a cross with a pure single race. Snowflake is a "double-throwing" variety, such as Miss Saunders (7) has shown to be permanently heterozygous, the pollen of the singles transmitting only doubleness while their eggs transmit singleness also. The average proportion of the sterile doubles from normal Snowflake parents has been about 53 per cent. To explain the usual excess of doubles over 50 per cent in such cases, Miss Saunders (7, 8) has postulated two complementary dominant genes for singleness, both heterozygous in double-throwers. To explain the absence of singleness from the functional pollen, the writer assumes the presence of a pollen lethal (3). Snow (9) states that Haldane has devised for the inheritance of doubleness a gamete-lethal scheme which fits the data remarkably well. Whatever is the best complete explanation for the single-double ratio, however, the

¹ Received for publication Nov. 19, 1925; issued July, 1926. Paper No. 138, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

² Reference is made by number (italic) to "Literature cited," p. 46.

³ When the account cited was written, it seemed probable that the Slender type depended on a mutant gene rather than on an extra chromosome. This view was based especially on the fact that two very dissimilar mutant forms, Slender and Crenate, both give apparent linkage ratios for doubleness. Both these forms, however, are trisomic; the somatic differences between them are doubtless related to a difference in the average length of the odd chromosome (6). Evidently the odd chromosome of Slender, at least, is a fragment.

⁴ Since doubleness obviously is a mutant character, the symbols D and d are used in place of S and s of an earlier paper (3). To indicate the pollen lethal about to be mentioned, D' might be substituted for D in the case of the double-throwers.

simple formulae given above seem adequate for the present purpose. Slender is known to carry an extra chromosome, hence a somatic variation which changes Slender to normal evidently involves the loss of that extra chromosome. If singleness changes to doubleness at the same time, plainly the lost chromosome carried some gene (D) essential to singleness.

The most significant new evidence is given by another change from Slender single to normal double, discovered in February, 1924, among progeny of a selfed Slender parent. In this case there is no room for doubt as to which was the original type of the plant, since the double flowers and normal leaves were confined to one main branch. The double flowers, as in the earlier case, suggested a chimera condition; usually there were four large petals, within



FIG. 1.—Flowers and leaves from the first plant described in this paper. At the right below, two rosettes with narrow leaves, from the Slender part of the plant; above each rosette, the corresponding raceme, with ordinary single flowers. At the left above, two intermediate or partially double flowers from the variant branch. Second from the left below, a fully double flower from a shoot borne by the variant branch. At the extreme left below, a shoot from the variant branch, with buds apparently fully double and with leaves broader and thicker than those of the Slender shoots

these there were smaller curled petals which sometimes bore traces of anthers, and in some flowers there was an abnormal pistil. One shoot, however, seemed to have ordinary double flowers.

In February, 1925, this plant was again in bloom, but it died before all the new shoots on the variant branch had produced well-developed flowers. All but one main branch was Slender single as before (fig. 1). On the variant branch, one shoot, with several racemes, bore ordinary double flowers, one of which is shown in Figure 1. Another shoot had buds which were probably also fully double. Most (apparently all) of the other five shoots produced abnormal intermediate flowers like those of the preceding season.

In this case and that previously reported, it would be expected that the intermediate flowers would be accompanied by leaves intermediate between Slender and normal, rather than by strictly normal leaves. On account of the great nongenetic variability of the leaves in general, it was not possible to determine this point. There was,

however, a marked change from the slender shoots and narrow leaves of the Slender form, in the direction of the normal type.

Another plant, from a Slender parent, had one vigorous variant branch with normal leaves and ordinary double flowers. The rest of the plant was very small, not as large altogether as the one variant branch; it bore extremely small leaves and single flowers. The single-flowered part in this case may have been Slender, dwarfed by the early development of a branch of the more vigorous normal type, or it may have been a weaker "secondary" trisomic type carrying an extra chromosome different from that of Slender.

In the latter case, presumably, a trisomic single plant produced a normal double by elimination of a chromosome which carried a gene necessary for single flowers. In the former case reported here the process doubtless was the same, except for extensive development of a chimeral condition. If the change in the former case had been due to the production, by gene mutation, of a new type intermediate between ordinary singles and ordinary doubles, the later occurrence of a second mutation changing the new type to ordinary double would have been a remarkable coincidence—unless the intermediate gene was exceptionally unstable. Further, if any of the three bud variations mentioned above arose by gene mutation, the simultaneous disappearance of the trisomic characters would be extremely improbable. Therefore, without having chromosome counts from these plants, one may be very confident that the cytological change consisted in the loss of a chromosome carrying a gene necessary for single flowers.

Another little plant of Slender parentage, itself apparently Small leaved (formerly Small, smooth leaved) (4), bore flowers that were all intermediate between singles and normal doubles. It presumably was a chimera, but the fertilized egg which produced it may have carried two extra chromosomes, one of which was later lost.

Still another plant with intermediate flowers throughout came from pollination of pure single by Slender double-thrower. Considered in connection with the first case described in this paper, it is highly probable that this plant was a single-double chimera. Since, however, the leaves appeared strictly normal, while the flowers were far from being fully double, it is possible that the double component of the chimera was produced by gene mutation, or at least in some other way than by nondisjunction of a whole chromosome. If this plant was originally a normal heterozygous single (dD) with 14 chromosomes, and the variant tissue was double (dO) with 13 chromosomes, the loss of a chromosome had surprisingly little effect on leaf form and vigor of growth. A *Datura* form (1) lacking one chromosome in its hypodermal layer differed only slightly from the normal diploid type, but another form, more abnormal, also lacked the same chromosome, and this difference, it is suggested, may have been due to a chimeral condition in the former case.

The flowers of this *Matthiola* form were highly variable, but, typically, they had two successive sets of floral parts from sepals to stamens (fig. 2). The stamens of the first set were more or less petaloid. Sometimes there was a recognizable pistil at the center of the flower, but often, at least, the axis was further proliferated. During several months of vigorous flowering, no ordinary flowers, either double or single, were observed.

The third new case of obvious somatic variation (if the two plants just described are to be classed as doubtful) occurred in a plant which as a young seedling seemed to belong to the Smooth-leaved trisomic type; this plant came from the parentage Smooth \times normal. Smooth is a trisomic form which (fig. 3) is usually smaller and later in flowering than the normal type of the same general genetic constitution. When in bloom this plant appeared normal in vigor, leaf form, and earliness. It was discovered, however, that the plant consisted mainly of one large branch of normal type, while the remainder was unquestionably Smooth. Smooth does not give trisomic ratios for doubleness, and both parts of the variant plant had double flowers. Here again is a somatic variation clearly dependent on elimination of a chromosome.



FIG. 2.—On the left, two racemes from an F_1 plant resulting from pollination of a pure single by a Slender (trisomic) double-thrower, showing normal single flowers and capsules. On the right, two racemes from a sib of the plant just mentioned, showing abnormal double flowers, the lower ones proliferating, as in ordinary doubles, to form branch racemes

DISCUSSION

As the bud-variation type in all these cases had sterile flowers, no genetic test was possible, and counting of somatic chromosomes has not been attempted. In *Datura* (1), however, where trisomic and tetraploid types have been identified as bud variations on normal plants, genetic tests have given positive results, and in another bud-variation form of *Datura* the loss of one chromosome has been cytologically demonstrated.

The fact that most of the variations mentioned in this paper involve flower form may be due to the definite and conspicuous nature of these flower characters. Changes similar to that in the last case would be much more likely to be overlooked entirely, or to be recorded as doubtful.

It is probable that changes from trisomic to normal in *Matthiola* are much more likely to become visible than are changes in the

reverse direction, even if the two classes of change occur with equal frequency. When trisomic and normal cells are present in the same plant, the greater vigor of the normal type would doubtless favor its predominance in early stages, as it so strikingly did in later stages in some of the plants described here. In fact, most of the variations described here may have been due to simple somatic nondisjunction of an odd chromosome, producing daughter cells with respective complements of 14 and 16 chromosomes. For, while the vigor of the normal 14-chromosome cell would favor its multiplication, the decided weakness of the tetrasomic type might be expected to lead to its elimination.

These cases add a little to the evidence in favor of Emerson's (2) conclusion that recognizable bud variations seem, in general, to be



FIG. 3.—F₁ progeny from a cross between a trisomic form (large-leaved) and a normal pure single. At the left, a Smooth-leaved trisomic mutant, smaller and later than the normal in the middle. At the right, a large-leaved trisomic, also late

more often due to unequal mitosis than to gene mutation. In view of the probability of severe selective elimination of forms with abnormal numbers of chromosomes, it is likely that bud-variation types actually originate far more often in the former way. Gene mutation is, of course, much handicapped in the production of visibly variant types, by the ineffectiveness of recessive mutation in material homozygous for the gene affected. It is possible, however, that somatic elimination of chromosomes is more frequent in trisomic plants than in normals.

Three of the *Matthiola* bud variations give a little evidence for the chromosome theory of heredity, evidence distinct from that given by the discovery of the association between extra chromosomes and mutant somatic characters. The simultaneous disappearance of singleness and trisomic characters suggests that singleness depended on the chromosome which was lost, and this conclusion is strongly reenforced by the fact that Slender parents give single-double progeny ratios indicating the presence of three allelomorphs (Ddd) for these characters.

Evidence on the nature of bud variation in annual plants is valuable not only as a contribution to general genetic theory, but also for the suggestions it may give as to the probable mode of origin of bud variations in clonal varieties, such as those of Citrus fruits.

SUMMARY

In four cases—three of them described for the first time in this paper—a trisomic, or (in one case) evidently trisomic, type of *Matthiola* has undergone somatic change to or toward the normal diploid type. In three of these plants the loss of trisomic characters was accompanied by change from single to double flowers. In two plants a periclinal single-double chimera evidently was formed, as was indicated by the intermediate condition of the double flowers and by a further change in one plant to fully double flowers.

Loss of a chromosome evidently was responsible for these bud variations, and probably also for the production of intermediate flowers in two other plants.

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THE PEA APHID AS AN ALFALFA PEST IN KANSAS¹

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INTRODUCTION

During the last decade the pea aphid, *Illinoia pisi* (Kalt), has become a major insect pest of alfalfa in Kansas. The first known outbreak occurred during the spring of 1921, and was rather general over Kansas and neighboring States. This aphid has long been known as an enemy of peas and less commonly of red and crimson clovers. Apparently it is extending its range of food plants, and bids fair to continue to be one of our most destructive alfalfa insects. This paper is based on observations of three outbreaks of this aphid, over a period of five years.

Some of the most important writings on the pea aphid are those of Sanderson (6)², Chittenden (1), Folsom (4, p. 138-154), Davis (2); and Smith (7). Published work has dealt with this insect almost exclusively as a pest of peas and clovers. This account is based entirely on rearings and field observations on alfalfa.

OUTBREAKS AND DISTRIBUTION IN KANSAS

The first outbreak in the spring of 1921 followed a mild, dry winter and an early advent of spring (figs. 1 and 2). The temperature at Manhattan was somewhat above normal, while the rainfall was below normal. In the fall of 1920, pea aphids were very abundant in some fields at Manhattan until the alfalfa was killed by frosts early in November. The spring of 1921 set in unusually early. There was a period of typical spring weather in February, and the aphids, already in the alfalfa, multiplied rapidly. New growth of alfalfa appeared rather slowly, as rain was badly needed. By early March the alfalfa over much of the State was heavily infested. Many fields made very little growth. On March 27 there was a very severe freeze, the temperature dropping to 18° F. The new growth of alfalfa was frozen, but the aphids were not perceptibly affected by the freeze. They clustered on the new growth and living parts of the plants in such numbers that it was impossible for the alfalfa to grow. As a result of the infestation and the late freeze, about 100,000 acres of alfalfa in Kansas were killed.³

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² Reference is made by number (italic) to "Literature cited," p. 57.

³ Secretary Mohler of the Kansas State Board of Agriculture (5), estimated the loss for that year as follows: "The belated first cutting of alfalfa is estimated at 650,000 tons, or slightly less than six-tenths of a ton to the acre. The first cutting last year amounted to 1,271,000 tons, or 1.01 tons to the acre. The damage to alfalfa by spring freezes and attacks of the pea aphid is clearly shown, not only in this exceptionally low yield, but in the impaired vitality of the plant as indicated by its slow recovery through the late maturity of the first cutting. Added to this is the fact revealed by the assessors' returns as of March 1, that the alfalfa acreage has decreased by 128,000 acres, and a further, though as yet undetermined, loss has been sustained since as a result of unseasonable freezing and depredations of insects."

The aphids disappeared rather suddenly. On April 11, 1921, the alfalfa was still heavily infested, but on April 22 only a few aphids could be found. An increasingly large number became alate and flew to other fields, permitting the alfalfa to get a start. The aphids were present in very small numbers in local fields during the remainder of the year. They were locally plentiful, especially on young alfalfa, during the fall of 1921 and the spring of 1922 in several places in the Kaw Valley, though very scarce at Manhattan. The damage, except in a few small areas, was not serious, however, for heavy spring rains brought on a luxuriant growth and at the same time checked the aphids. The aphids were present in local fields, however, until the middle of June and then disappeared. During November they were

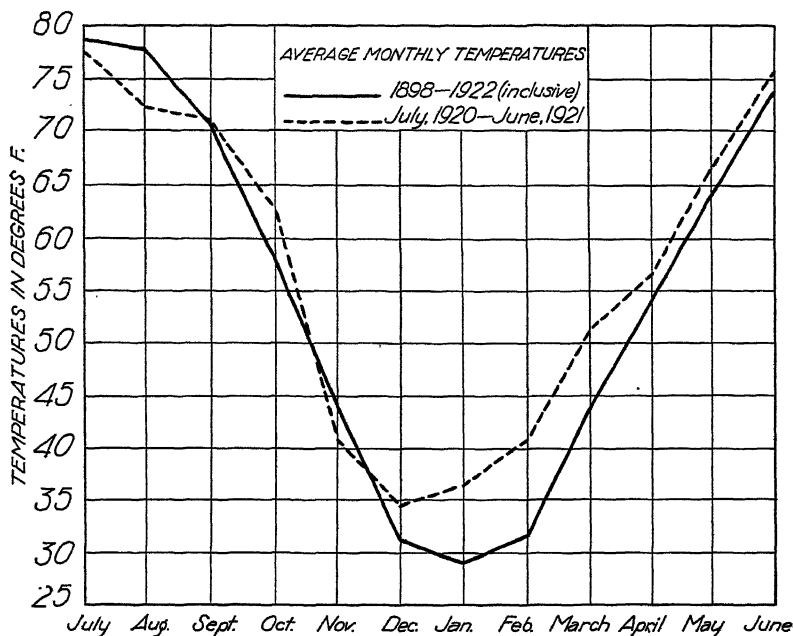


FIG. 1.—Average monthly temperatures for the 25 years, 1898-1922, inclusive, and for the 12-month period from July, 1920, to June, 1921, inclusive, at the Kansas State Agricultural College, Manhattan, Kans.

searched for at Manhattan with especial diligence, but only two were found.

In the spring of 1923, local outbreaks again occurred in the Kaw Valley from Wamego to Kansas City. There were also small, scattered infestations elsewhere in the State. Often only small areas in fields showed injury, but occasionally an entire field was completely killed. Small numbers were observed in fields near Manhattan during the fall of 1923, but they were more abundant in fields between Manhattan and Topeka.

A small and restricted outbreak occurred at Manhattan during April and May, 1924. The insects were present at the same time in small numbers over the larger part of Kansas. Some injury occurred to fields at Lincoln, Kans., and at different points in the Kaw Valley.

A few aphids were observed in alfalfa fields every week of the summer. Small areas were found at Manhattan during the fall of 1924 where they were fairly plentiful, though not doing injury.

SEASONAL HISTORY

The pea aphid is present during the entire year in alfalfa fields. On alfalfa at Manhattan it overwinters chiefly as an apterous viviparous female, and sometimes in the egg stage also. The adults survive best in winter in low areas and more protected parts of fields. Areas protected by hills, buildings, or trees seem to be especially favorable. Heavy growths of alfalfa left to freeze in the fall also seem to be protective.

Though careful searches were made for eggs, none was discovered at Manhattan until the autumn of 1924, when they were found in considerable numbers on the upper and lower surfaces of leaves and on stems of alfalfa plants.

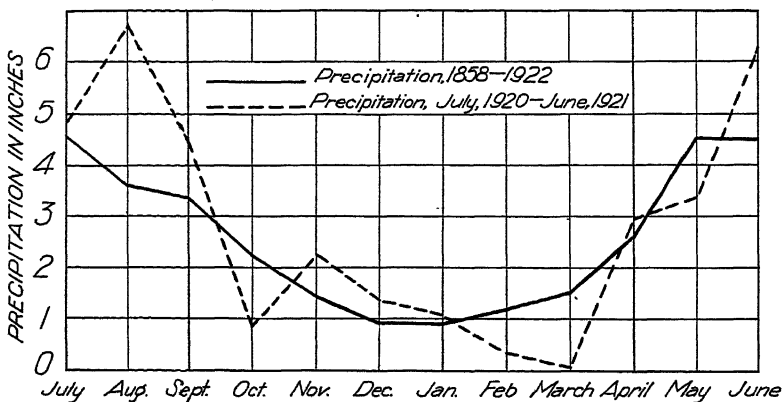


FIG. 2.—Average monthly precipitation for the 65 years, 1858-1922, inclusive, and for the 12-month period from July, 1920, to June, 1921, inclusive, at the Kansas State Agricultural College, Manhattan, Kans.

HOST PLANTS AND INJURY TO THEM

Alfalfa and sweet clovers (*Medicago*) and white and crimson clover (*Trifolium*) have been recorded as host plants for pea aphids. The aphids also have been taken repeatedly on vetch at Manhattan. After a spring outbreak they become numerous on both common garden peas and sweet peas. Winged forms have been taken on many other plants, but they were not observed to reproduce upon them.

The injury to alfalfa is distinctive, and a heavily infested spot may be discerned at a considerable distance. The plants are short and bunched, the tops light green in color, and the lower leaves yellow or dead, the general appearance from a distance being brownish. The bare ground shows up from lack of covering, and it is usually heavily besprinkled with the whitish aphid skins or molts. The aphids which cluster on both the stems and under side of the leaves show a preference for the younger growth. If the infestation continues, the leaves wither and the plants eventually die. The aphids leave, how-

ever, before the plants are dead. These aphids have been observed feeding or attempting to feed on wild mustard, shepherd's purse, and other weeds, when driven to it by the death of alfalfa. The alfalfa acreage in Kansas has decreased from 1,360,000 acres in 1915 to 884,000 acres in 1924, and the pea aphid has been an important factor in this decrease.

WINGED AND WINGLESS FORMS

The percentage of winged forms gradually increases as spring advances. Observations indicate that as they become crowded on the alfalfa plants, or that as the plants begin to weaken an increasingly large number of progeny is winged, thus enabling them to scatter. However, this condition may be partly cyclical, also.

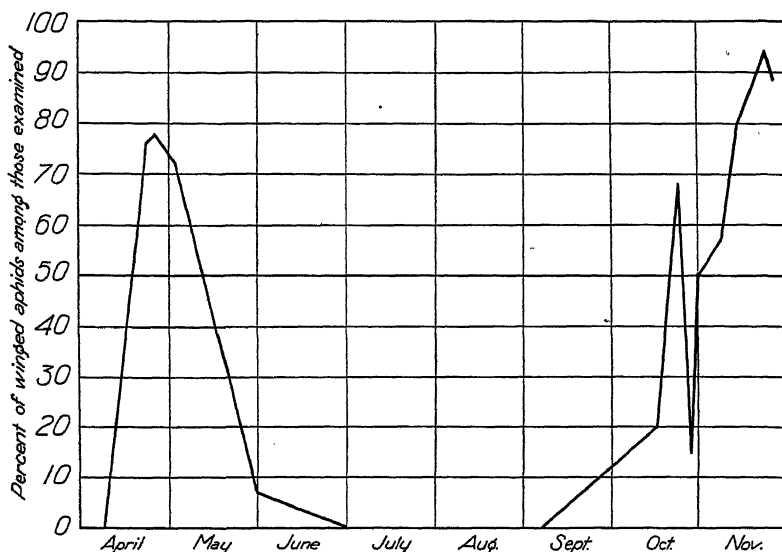


FIG. 3.—Percentage of aphids that were winged among those that were examined in various months of 1924

Field collections of pea aphids were made every week during the summer of 1924, beginning April 8. The number taken varied because of scarcity in the fields, but whenever possible, 300 was the number counted. Only winged adults or apterous adults in which the eye spots of their young could be seen in their abdomens were counted. The results of these counts for one year are indicated in Figure 3. This graph shows that in the early spring this aphid was predominately wingless. Some time between April 8 and 24 the winged forms equaled and on April 24 exceeded the wingless forms. On May 5 the winged forms reached their maximum and then decreased. Only wingless forms were found during the summer. In the fall the winged forms increased in an irregular manner until in November they again predominated. There were not sufficient aphids during the spring and summer of 1925 to repeat these counts.

CLIMATIC INFLUENCES

The numbers and activity of the pea aphid in the field are controlled very largely by climatic conditions. The outbreaks thus far observed indicate that this insect thrives best when the winter is mild and relatively dry. Early rapid growth of alfalfa, especially in March and April, greatly reduces or eliminates the possibility of serious damage by this insect. The three outbreaks followed a warmer and drier February than is the average, and March had a somewhat reduced precipitation and was warmer than the average. The aphids reproduce slowly at a lower temperature than that in which ladybird beetles become active. Temperatures of 90° to 100° F. are clearly unfavorable, especially when accompanied by low humidity. A temperature of 100° or higher was usually quickly fatal in the temperature-control cages. This agrees in the main with the conclusions of Smith (?), though the temperature and humidity optimum of Virginia is apparently somewhat higher than in Kansas.

The well-known fungous disease which develops under humid conditions and which has been reported frequently as being the most important natural control of this aphid, has been less important during these outbreaks than predacious insects, especially coccinellids.

REARINGS IN INSECTARY AND FIELD

Studies on the general biology of the pea aphid were begun in October, 1923, and continued, as material and temperature permitted, until the fall of 1924. The usual lamp-chimney cage over young growing alfalfa plants was used. One series (Y) was reared under temperature-controlled condition and another series (Z) was reared under ordinary greenhouse conditions. The rearings were transferred to a small field laboratory in April, 1924, where the work was carried on during the summer. These results are indicated here as the F series.

Each of the series was started by bringing in from the field a mature female and placing her on alfalfa. Her progeny was transferred to other alfalfa plants as produced, and were numbered Y2 or Z2, etc. A summary of the rearings under the uncontrolled-temperature or ordinary greenhouse conditions is given in Table 1.

In the fall of 1924, rearings were conducted in the greenhouse in the same manner as those shown in Table 1, as a check on the work of 1923. The numbers of young and length of reproductive period of the aphids in this series were slightly greater than for those recorded in Table 1. This may have been due to two conditions: (1) In 1923 the weather was too severe for the aphids to reproduce in the field during the time the experiment was being carried on in the greenhouse, while in 1924 the aphids were reproducing in the field the entire time the experiment was being carried on in the greenhouse, and (2) the technique had been improved, so that it may have been that better care was given to those of the 1924 series.

The averages for the 116 individuals studied in both series in the two years are as follows: The average age of female when first young was born, 9.7 days; the youngest age, 6 days. The average length of reproductive period was 12.8 days; the longest, 31 days.

The average number of young produced was 39.3; the largest number, 93. The average length of life was 25.1 days; the longest life, 43 days.

TABLE 1.—*Studies of life histories of pea aphids under greenhouse conditions, 1923-24*

Series Z		Age of female when first young was produced (days)	Days of reproductive period	Number of young	Largest number of young per day	Date of death	Length of life	Temperature			Humidity, average hourly
Generation	Date of birth							Maximum	Minimum	Average	
								°F.	°F.	°F.	
1 ^a	Nov. 9	7	6	23	5	Nov. 22	13	97	65	77	75
2.....	Nov. 16	13	4	15	5	Dec. 3	17	100	63	77	73
3.....	Nov. 22	8	2	12	11	Dec. 2	10	100	63	77	73
5.....	Dec. 1	7	5	14	5	Dec. 13	12	97	65	76	78
6.....	Dec. 9	11	8	20	4	Dec. 28	19	100	70	79	77
7.....	Dec. 20	8	6	20	4	Jan. 3	14	96	70	78	80
8.....	Dec. 27	8	6	18	9	Jan. 11	15	95	60	76	80
9.....	Jan. 4	10	5	19	8	Jan. 19	15	97	60	77	79
10.....	Jan. 13	8	3	11	6	Jan. 24	11	97	65	79	79
11.....	Jan. 21	11	18	47	8	Feb. 20	30	97	60	79	80
12.....	Feb. 1	7	12	53	11	Feb. 20	19	95	61	79	78
13.....	Feb. 9	7	31	58	6	Mar. 25	45	97	61	78	80
14.....	Feb. 17	8	16	84	14	do.	37	97	61	78	80
15.....	Feb. 25	8	20	69	13	Mar. 31	35	98	61	77	76
16.....	Mar. 5	6	13	78	14	Apr. 7	33	98	60	76	75
17.....	Mar. 11	9	18	77	14	Apr. 8	28	100	62	76	77
18.....	Mar. 20	7	16	93	14	Apr. 12	23	100	60	76	80
19.....	Mar. 27	9	8	44	7	Apr. 14	18	100	60	77	81
20.....	Apr. 5	7	5	24	11	Apr. 18	13	100	60	79	80
21.....	Apr. 12		(^b)								
Average of 19 individuals.....		159	202	779			407				
		8.3	10.6	41			21.4				

^a Caught wild, record incomplete.

^b Died.

Folsom (4, p. 138-154), recording the work of Webster, gives the following averages for 16 life histories reared in a line on peas from March to September: The average number of days to maturity, or the beginning of reproduction, was 11; length of life, 25.4 days; average number of young, 46. Davis (2) obtained the following averages: Age at which females reproduce "varied from 6 to 35 days, with an average of 10.3 days, from 38 records covering the months from May 15 to December." Average of all rearings over two years was 12.1+ days; length of reproductive period varied from 2 to 68 days, or an average for 53 examples over two years of 22 days; total length of life, 10 to 85 days, or an average of 39.1+ days over two years; average total number of young produced was 68.3, with one instance of 124 as a maximum.

The results of the three series of rearings reported in this paper differ from the above results chiefly in that the Kansas aphids began to reproduce after a slightly shorter post-natal period and their reproductive period and their average length of life were both somewhat shorter also.

The experiments the results of which are recorded in Table 2 were carried on in one of the temperature-controlled chambers described by Dean and Nabours (3). The temperature shows a

rather wide range from maximum to minimum, but these extremes were in the form of peaks and remained at these points only a few minutes. The averages of the different phases of the life cycle recorded here show a decided increase over those recorded in the preceding table. The number of days of the reproductive period in the constant-temperature cages was 16.4 days, compared to 10.6 days in the greenhouse. The average number of young in this experiment was 54.3 in contrast with the 41.0 in Table 1. The length of life was also longer in the controlled temperature. The largest number of young from one female was 121 (Table 2), which occurred during the time when the average temperature was 65° F. The temperature in the experiment at the start was above 70°, and continued to be that for about a month. When the average temperature was decreased to 65° there was a decided increase in the number of young produced. A decrease in the number of young per individual occurred when the temperature was again raised to 70°. This decrease, however, was not as marked as the increase resulting from lowering the temperature from 70° to 65°. The differences may have been due to the conditions under which the aphids were kept during nymphal development. It seems probable that the optimum temperature for this aphid is between 60° and 70°, though no definite conclusion can be drawn, for it was impossible to hold the temperature for any length of time at points below 60°. Hence, no data on lower temperatures are available.

TABLE 2.—*Life histories of pea aphids under controlled temperature conditions, 1924*

Series Y		Age of female when first young was produced (days)	Days of reproductive period	Number of young	Largest number of young per day	Date of death	Length of life	Temperature			Humidity, average
Identification No.	Date of birth							Maximum	Minimum	Hourly average	
								°F.	°F.	°F.	
8 (1).....	Jan. 4	9	2	12	6	Jan. 15	11	83	70	75	74
9 (3).....	Jan. 13	7	8	39	7	Jan. 28	15	80	60	70	75
10 (1).....	Jan. 20	10	19	19	7	Feb. 17	28	80	60	70	78
11 (1).....	Jan. 30	11	8	17	3	Feb. 17	19	80	60	70	78
12 (1).....	Feb. 10	11	24	75	7	Mar. 21	40	80	57	65	78
12 (2).....	Feb. 10	10	19	98	10	Mar. 20	39	80	57	65	78
13 (2).....	Feb. 20	10	18	121	11	Mar. 29	38	80	55	65	78
14 (1).....	Feb. 29	9	30	87	12	Apr. 8	39	90	57	70	81
14 (2).....	Feb. 29	11	20	68	7	Apr. 8	39	90	57	70	81
15 (1).....	Mar. 9	9	21	90	12	Apr. 8	30	87	55	70	81
15 (2).....	Mar. 9	10	19	97	9	Apr. 8	30	87	55	70	81
16 (1).....	Mar. 19	8	12	64	14	Apr. 8	20	85	55	70	70
16 (2).....	Mar. 19	8	10	65	10	Apr. 8	20	85	55	70	80
17 (1).....	Mar. 28	8	7	35	9	Apr. 16	19	85	55	70	81
10 (2).....	Jan. 13	8	5	14	5	Jan. 26	13	83	70	70	76
11 (2).....	Jan. 21	8	16	29	7	Feb. 28	38	80	60	70	78
11 (3).....	Jan. 27	12	19	22	4	Feb. 27	31	80	60	70	78
11 (4).....	Jan. 29	12	17	20	3	Mar. 5	35	80	57	70	81
11 (5).....	Jan. 29	12	7	12	4	Mar. 13	43	80	57	70	81
12 (1).....	Jan. 29	10	7	17	5	Feb. 15	17	80	60	70	81
12 (2).....	Jan. 29	10	32	55	9	Mar. 10	42	80	55	65	80
13 (1).....	Feb. 8	9	13	51	6	Mar. 3	24	80	57	70	80
13 (2).....	Feb. 8	10	32	67	5	Mar. 24	45	80	57	65	81
14 (1).....	Feb. 17	11	26	111	10	Mar. 25	37	80	55	65	80
14 (2).....	Feb. 18	10	26	95	7	Apr. 8	50	90	55	70	80
14 (3).....	Feb. 17	12	28	75	7	Apr. 7	50	90	57	70	81
14 (4).....	Feb. 18	11	11	54	9	Mar. 13	24	80	55	70	80
15 (2).....	Feb. 28	9	8	30	6	Mar. 6	18	90	57	70	80
16 (2).....	Mar. 9	8	12	53	9	Mar. 29	20	87	55	70	80
17 (1).....	Mar. 18	8	16	37	6	Apr. 15	28	85	55	70	80
A. v. of 30 individuals.....		9.7	16.4	54.3	-----	-----	30	-----	-----	-----	-----

The experiments the results of which are recorded in Table 3 were carried on in the field laboratory during the summer of 1924. It was difficult to rear the aphids over a complete life cycle. However, the aphids were also decreasing in numbers in an adjoining alfalfa field, so the insectary equipment apparently was not responsible for the difficulties encountered in the rearing in the insectary. Attempts during the summer of 1925 to rear an unbroken line through the summer on alfalfa at the field insectary were unsuccessful.

A comparison of the data obtained under field-insectary and greenhouse conditions is of interest. The average age of the female when first young was born in the field insectary was 10.6 days, and in the greenhouse it was 8.3 days. The average length of the reproductive period in the field insectary was 3.3 days, and in the greenhouse it was 10.8 days. The average number of young produced in the field insectary was 13.4 and in the greenhouse 21.4. In the field insectary, as well as in the greenhouse, the largest number of young was produced when the average temperature was about 60 or 65° F. Also, the aphids lived longest at these temperatures. However, under both of these conditions the averages were not nearly so high as when the temperature was more constant. In the greenhouse, where the variation in temperature was greater than that in the air chambers, the averages of the life activities of the aphids were smaller.

TABLE 3.—*Life histories of pea aphids under field-insectary conditions, 1924*

Series F		Age of female when first young was produced (days)	Days of reproductive period	Number of young	Largest number of young per day	Date of death	Length of life	Temperature			Humidity, approximate average
Identification No.	Date of birth							Maximum	Minimum	Average	
2 (8).....	June 30	10	2	6	3	July 12	12	98	46	74	72
2 (1).....	May 1	20	1	2	2	June 1	31	90	35	60	71
3 (1).....	May 19	12	10	66	7	June 13	25	95	35	65	81
3 (2).....	May 19	12	7	42	10	June 9	21	95	35	62	80
4 (1).....	May 30	9	8	23	8	June 16	17	95	47	75	79
5 (1).....	June 8	8	5	13	6	June 23	15	104	60	80	79
6 (2).....	June 16	10	2	3	2	June 30	14	104	58	80	80
7 (1).....	June 26	12	1	2	2	July 9	13	100	46	75	75
2 (1).....	May 5	18	1	9	9	June 1	27	90	35	60	70
3 (1).....	May 23	12	3	19	7	June 9	17	95	35	65	70
3 (2).....	May 23	12	2	9	5	June 9	17	95	35	65	70
4 (1).....	June 4	8	3	11	7	June 17	13	95	47	75	80
4 (2).....	June 8	7	3	12	5	June 19	11	104	60	80	80
2 (1).....	June 24	12	8	20	5	July 15	21	100	46	75	80
2 (2).....	June 24	10	2	8	4	July 8	14	100	46	75	75
2 (3).....	June 24	10	3	11	4	July 9	15	100	46	75	75
3 (6).....	July 7	8	1	10	10	July 17	10	104	55	80	70
3 (8).....	July 7	8	1	8	8	July 17	10	104	55	80	70
2 (1).....	June 30	10	6	15	4	July 17	17	104	55	77	73
2 (2).....	June 30	11	1	3	3	July 12	12	97	55	75	73
2 (3).....	June 30	11	2	4	2	July 13	13	97	55	75	73
2 (5).....	June 30	10	3	10	8	July 13	13	97	55	75	73
2 (14).....	July 2	10	1	3	3	July 14	12	97	55	77	70
Av. of 23 individuals.....		10.8	3.3	13.4	-----	-----	16	-----	-----	-----	-----

CORRELATION RATIOS

Correlation ratios and probable errors were computed for several factors of the life cycle of the pea aphid. The averages from Tables 1 and 2 were used. The formula used for the correlation computation was:

$$r = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{N}}{\sqrt{\left[\Sigma X^2 - \frac{(\Sigma X)^2}{N} \right] \left[\Sigma Y^2 - \frac{(\Sigma Y)^2}{N} \right]}}$$

X and Y are the original numbers, and N is the total number of individuals. The probable error was computed by the following formula, r being the correlation ratio:

$$\pm \frac{0.6745 (1 - r^2)}{\sqrt{N}}$$

When the correlation is 4.9 times the probable error, the results are regarded as significant; that is, according to the laws of chance, such condition would happen only once in 1,052 trials.

These determinations were made to show the presence or absence of correlations, not only between the different factors of the life cycle of the pea aphid, but also between these factors and the average hourly temperature. These determinations are given in Table 4.

TABLE 4.—*Correlation between various factors in the life cycle of pea aphids*

Between	Data from Table 1		Data from Table 2	
	Correlation coefficient	Probable error	Correlation coefficient	Probable error
Age of mother when first young was born and—				
Length of reproductive period.....	−0.1695	±0.1503	0.3522	±0.1079
Total number of young.....	−.3042	±.1404	.06681	±.1226
Length of life.....	−.1006	±.1532	.5583	±.0848
Average hourly temperature.....	.1854	±.1494	−.227	±.1168
Length of reproductive period and—				
Total number of young.....	.7588	±.06559	.6209	±.0766
Length of life.....	.9324	±.02083	.8242	±.0395
Average hourly temperature.....	−.01248	±.1547	−.5971	±.07926
Total number of young and average hourly temperature.....	−.2288	±.1466	−.5624	±.08426
Length of life and average hourly temperature.....	−.0092	±.1547	−.5277	±.08822

The significant correlations are (1) between the age of the mother when the first young was born and the length of life under controlled conditions; (2) between the length of the reproductive period and the total number of young produced; (3) between the length of reproductive period and the length of life; (4) between the length of the reproductive period and the average hourly temperature in the controlled temperature series; (5) between the total number of young and the average hourly temperature in the controlled temperature series; and (6) between the length of life and the average hourly temperature in the controlled temperature series.

The first three correlations will not be discussed here, for the reason that they are either readily explainable or not important in this discussion. In the latter three correlations, it seems clear that the length of the reproductive period, the total number of young produced, and the length of life of the mother are strongly affected by temperature. It should be remembered that food conditions were kept at or near the optimum, and that the variations in humidity were slight. The data indicate that as the temperature varies from the indicated optimum, the length of life, the length of reproductive period, and the total number of young produced are reduced.

The average length of life under uncontrolled temperatures was 21 days, and the number of young produced was 41, while corresponding data under controlled temperature was 30 days and 54 young.

The largest number of young was produced at an average temperature of 65° F. and a humidity of 80°. As the average temperature increased, the number of young produced decreased. This is especially important in explaining the seasonal activity of these aphids in the field. The aphids are most abundant and injurious in the field in April and May. The 37-year average temperatures for these months for the State are 54.6° and 63.6° F., respectively, which are not far from the indicated optimum.

The aphids become very scarce in early June, and remain so until September. Rearings in the field insectary, as recorded in Table 3, emphasize the reduced rate of reproduction and shortened life, which, with the activities of natural enemies and the cutting of the crop, explain their scarcity in the field. It was almost impossible to keep an unbroken series going in the field insectary during the summer, under the most favorable food conditions, and where natural enemies and the cutting of the alfalfa were not influencing factors. This would indicate that the high summer temperature and low humidity were dominant influences.

In September and October the average temperature approaches the optimum for this insect, and the aphids again become fairly plentiful though not particularly injurious. The average temperatures for these two months for 1920, when aphids were unusually plentiful in certain fields at Manhattan, were 69.6° and 60.8° F., respectively.

SUMMARY

During these studies, three outbreaks of the pea aphid—one of major importance, and the other two of minor importance—occurred in alfalfa fields in Kansas. Observations were made upon the aphid's behavior in the field and upon some features of its life economy, by rearings on alfalfa in a field laboratory, in the chambers of a controlled-temperature apparatus, and in an ordinary greenhouse. The following are the results of the three series of rearings:

Series F: Twenty-three individuals reared on alfalfa in field laboratory; average temperature from May 1, 1924, to July 14, 1924, 73° F.—range 35° to 104°.

Series Y: Thirty individuals reared on alfalfa under controlled-temperature conditions; average temperature from January 4, 1924, to April 15, 1924, was 69° F.—range 55° to 90°.

Series Z: Twenty-one generations reared on alfalfa in greenhouse, under natural greenhouse conditions; average temperature from November 9, 1923, to April 18, 1924, was 77.4° F.—range from 60° to 100°.

TABLE 5.—*Summary table*

	Series F			Series Y			Series Z		
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum
Age of female when first young was produced (days).....	10.8	20	7	9.7	12	7	8.3	13	6
Length of reproductive period (days).....	3.3	10	1	16.4	32	2	10.8	31	3
Number of young.....	13.7	66	2	54.3	121	12	41	93	11
Average number of young per day of reproductive period.....	3.9	10	1	3.3	6	1	3.7	6	2
Length of life (days).....	16.0	31	10	30.0	50	11	21.4	45	10

The optimum temperature for this aphid seemed to be at or near 65° F., at a humidity of about 80 per cent, but the exact optimum could not be determined. The aphids were most plentiful in the field during the months which have an average temperature near 65°—viz, April, May, September, and October. There is a strong indication that the rainfall of March is a governing factor in Kansas, with respect to possible damage to alfalfa. It was difficult, and in some cases impossible, to maintain an unbroken line in the field insectary during the summer. The aphids are very scarce in alfalfa fields also at this time. There is an indication that the high summer temperatures and low humidity are predominating influences, although natural enemies are also a factor. The pea aphids overwinter at Manhattan chiefly as nymphs and adults, but eggs were taken during the winter of 1924.

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CLIMATIC EFFECTS IN THE METABOLISM OF THE SUGAR BEET¹

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INTRODUCTION

In the summer of 1919 the senior writers undertook an investigation of nitrogenous metabolism in leaves of the sugar beet, which has been reported briefly (17, p. 17).³ It was the purpose of that work to determine whether protein synthesis occurred simultaneously with carbon assimilation or ensued in the following period of darkness. The results of earlier investigation on this point indicate the first of these relations. Kosutany (11) found a little greater percentage of soluble protein in leaves of *Vitis riparia* at 3 a. m. than at 3 p. m. On the other hand, Suzuki (22), employing leaves of several plant species, found at 6 p. m. a decided excess of this constituent over the percentage at 6 a. m. Schulze and Schütz (18), basing results upon both the number of leaves sampled and the weight of fresh material, concluded that with younger leaves of boxelder (*Acer negundo*) proteins migrated at night from the seat of synthesis. Similarly, Pigorini (16) found a nocturnal decline of more than 11 per cent in the protein nitrogen of leaves of mulberry (*Morus alba*). These results were based on weights of fresh tissue. According to Chibnall (?), this is the most accurate method of expressing the results. He found (8) with the runner bean (*Phaseolus vulgaris*) a decline of nearly 2 per cent of foliar protein nitrogen at night.

ANALYTICAL PROCEDURE

Inasmuch as preliminary tests (24) had indicated the impossibility of preserving the tissue without alteration of the soluble protein, the freshly sampled material was extracted directly. The leaf surface was dried by absorbent paper and brushed free of foreign material. After dissecting out the midribs the blade tissue was rapidly chopped fine, thoroughly mixed and replicate samples of 20 or 25 gm. were taken for moisture determination. Replicate samples of 10 gm. were used for determining total nitrogen. A 100-gm. portion of the tissue was triturated rapidly in a large porcelain mortar with the addition of an equal weight of washed, fine, spherical sand. About 5 c. c. of ether was added to promote plasmolysis, and water was added as required to give proper consistency for trituration. This took about 15 minutes. The pulverized tissue was then transferred

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² With the collaboration of H. Bernstein and N. T. Nelson, graduate students, University of Wisconsin, and H. B. Parmele and A. D. Dickson, assistants, Bureau of Plant Industry, United States Department of Agriculture.

³ Reference is made by number (italic) to "Literature cited," p. 75.

to a double layer of cheesecloth and extracted with successive portions of water to a volume of about 1,800 c. c., wringing out each portion of wash water. This extract was passed through a layer of paper pulp nearly 0.5 cm. thick on a Buchner funnel, with the aid of gentle suction. Petioles and roots were similarly treated. When washed to a volume of 2 liters, the filtrate was usually quite transparent and green to brown in color. Aliquots of 500 c. c. were brought quickly to boiling and coagulated with a few drops of 10 per cent acetic acid. This precipitate of the soluble protein was filtered out and determined by the Kjeldahl-Gunning method, the time required from sampling of the tissue to boiling of the extract being about one hour. This period is of importance in relation to a subsequent discussion of enzymatic action. The extent of extraction specified here had been found to remove all but traces of soluble alpha-amino nitrogen compounds.

The residual solution from the separation of soluble protein was employed for determination of sugars and of nitrogen present as amino acids and other forms. Separate aliquots from the 2 liters of filtered extracts served for the determination of total soluble nitrogen. Reducing sugars were determined by the Shaffer-Hartmann method (19), and sucrose by the Herzfeld process for inversion (5, p. 266).

It appears that the amount of nitrogen, apparently in the form of soluble protein, may depend upon the degree of grinding of the tissue, as well as upon the methods of filtering. The writers have found that this constituent is relatively independent of the thickness of paper pulp employed as a filter. Comparison of the composition of extract with that obtained by use of a mill for grinding, as employed by Osborne (14) with frozen tissue, is given in Table 1. The writers applied the mill to the fresh, unfrozen tissue.

Microscopic examination indicated that the walls of practically all cells were ruptured by the use of a mortar for grinding. The results show that the mill treatment recovered 10 to 15 per cent more of the total nitrogen in soluble form than did use of the mortar. This is accounted for largely by the increased dispersion of protein. That a similar effect can be obtained by regrinding the extracted tissue in the mortar method is indicated by preliminary tests. The other differences in composition of extract by the two methods of grinding are too small to appear significant.

TABLE 1.—Composition of extract obtained from sugar-mangold leaves by mortar and pestle, compared with use of the Nixtamal mill

[Values are percentages of dry matter]

	Sample A		Sample B	
	Mortar	Mill	Mortar	Mill
Total nitrogen.....	5.40	5.40	5.42	5.42
Soluble nitrogen.....	3.62	4.20	3.42	4.28
Coagulable nitrogen.....	2.70	3.28	2.40	3.16
Other soluble nitrogen.....	.92	.92	1.02	1.12
Alpha amino nitrogen.....	.35	.22	.31	.22
Reducing sugars.....	3.00	3.05	2.45	2.60
Sucrose.....	1.40	1.55	1.60	1.35

A phase of this process of recovering the soluble constituents which should receive special attention is the possibility of inversion of sucrose during the period between sampling and heating the extract for recovery of soluble proteins. To test this matter, extracts obtained by maceration in a mortar, in which the time elapsed until inactivation of enzymes by boiling did not exceed 30 minutes, were compared with those obtained from corresponding leaves killed immediately after sampling by immersion in boiling alcohol (13). CaCO_3 was added promptly to the extracts. The data appear in Table 2.

TABLE 2.—*Comparison of recovery of sugars from sugar-beet leaves by direct extraction with alcohol and delayed extraction with water*

[Values are percentages of dry matter]

	Time of sampling					
	Morning		Noon		Night	
	Direct	Macerated	Direct	Macerated	Direct	Macerated
Reducing sugars.....	2.85	2.90	4.74	4.83	1.40	1.49
Sucrose.....	1.70	1.71	1.35	1.43	2.00	2.10

These results show that no inversion occurred during the time required by the extraction method employed in the present investigation. Similar conditions were found to hold for petiole and root tissues. Preliminary trials over longer periods indicated that inversion of sucrose in leaf tissue does not become appreciable at room temperatures until after one hour. This gives good grounds for assuming that marked variations in the distribution of sugars in the present case existed in the tissues as sampled.

RESULTS OF 1919

Samplings from many plants were taken at about sunrise and sunset. Only two leaves were taken from a single plant, and these were intermediate in age to the foliage as a whole. The climatic data are those recorded at the weather station, located about 1.2 kilometers east of the beet field and at some 30 meters greater elevation than the beet field.

In Table 3 are assembled the climatic and analytical data of this period. A value of 20 per cent has been assumed to approximate the average nitrogen content of soluble compounds other than proteins, amino acids, and ammonia, reported here as "rest-soluble N." All of the values for nitrogenous compounds were based on the dry matter. In this particular case the values for sugar were obtained by the Defren-O'Sullivan method. Therefore they probably exceed the true reducing sugars, and are of questionable significance. The climatic and analytical data are assembled in Figure 1.

It appears from the graphs that the time of day was less significant than temperature in determining differences of composition. The limited and questionable data for reducing sugars seem to be directly related to temperature variations. The soluble protein varies in an inverse manner to changes of temperature, and with particularly

marked effect on the cold morning of August 27. This is true also of the other higher forms of soluble, assimilated nitrogen designated as "rest-soluble." The insoluble protein varies in the same directions as temperature, while α -amino nitrogen varies with temperature in the afternoon samplings, but inversely to it in the morning. Ammonia varies in the manner of temperature throughout, but amide follows the course of amino acid. For some reason not apparent, the data for the evening of September 6 are erratic throughout as compared with other dates. It may be noted that a medium degree of radiation coincides with a high temperature efficiency on this date.

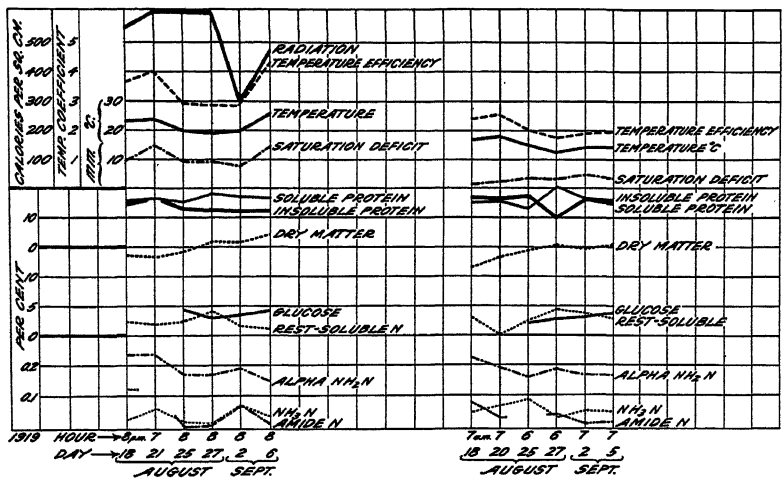


FIG. 1.—Graphs of climatic and analytical data of 1919 investigations of the leaf of sugar mangold

TABLE 3.—Climatic data and analytical results for investigation of sugar-beet leaves, 1919

Date	Climatic data				Dry matter	Analytical data of dry matter						
	Temperature	Temperature co-efficient	Radiation calor-ies per square centimeter	Vapor pressure deficit		Reducing sugars	Insoluble protein	Soluble protein	Rest-soluble N×5	α-Amino N	Amide N	Ammonia N
	° C.			Mm.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Aug. 18, 7 a. m.-----	17.5	2.5		1.9	11.9		16.9	14.6	2.9	0.22	0.08	0.05
8 p. m.-----	23.5	3.7	556	9.5	13.7		16.6	16.9	2.3	.24	.12	.03
Aug. 20, 7 a. m.-----	17.8	2.5		2.3	13.4		16.7	15.2	.2	.19	.03	.07
Aug. 21, 7 p. m.-----	24.4	4.0	597	14.3	13.6		17.3	17.4	2.1	.25		.06
Aug. 25, 6 a. m.-----	14.4	2.0		4.0	14.6	2.5	17.0	12.5	2.6	.16		.05
8 p. m.-----	20.0	2.9	592	9.4	14.6	4.1	13.5	15.6	2.5	.17	.01	.02
Aug. 27, 6 a. m.-----	12.8	1.8		3.1	15.2	3.3	9.8	20.5	4.8	.19	.04	.03
8 p. m.-----	19.4	2.8	585	9.2	16.1	3.2	12.0	18.5	4.1	.17	.02	.03
Sept. 2, 7 a. m.-----	13.9	1.9		5.2	14.9	3.2	15.5	16.3	3.3	.17	.02	.05
8 p. m.-----	20.0	2.9	284	7.8	16.1	3.4	11.8	17.9	1.5	.19	.06	.06
Sept. 5, 7 a. m.-----	14.4	2.0		2.8	15.3	3.6	15.2	15.0	3.2	.17	.02	.05
Sept. 6, 8 p. m.-----	26.1	4.5	480	14.1	17.3	3.9	12.4	16.3	1.0	.15	.02	.03

RESULTS OF 1920

The dates of sampling in this season were necessarily widely separated. This should be kept in mind in considering irregularities of the analytical data, particularly as regards the widely separated final date of September 29. The data are given in Table 4, and are shown graphically in Figure 2.

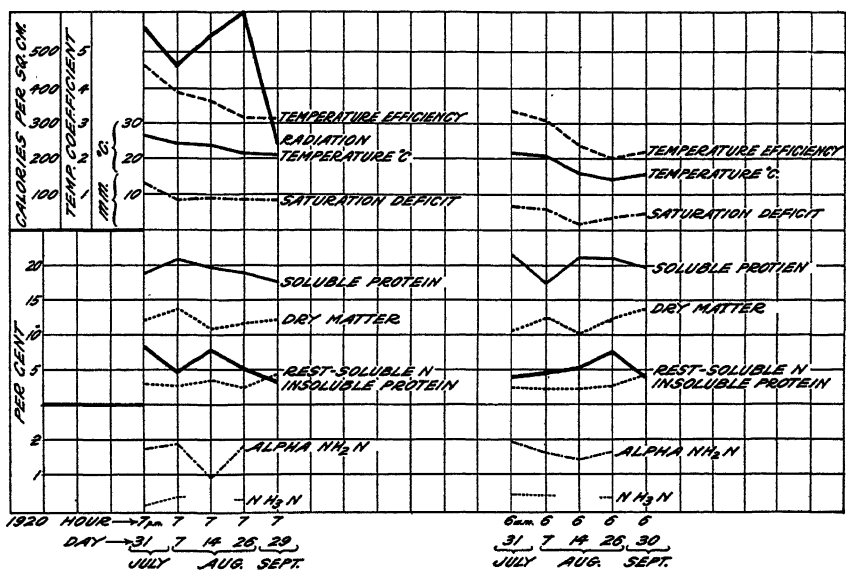


Fig. 2.—Graphs of climatic and analytical data of 1920 investigations of the leaf of sugar mangold

TABLE 4.—Climatic data and analytical results for investigation of sugar-beet leaves, 1920

Date	Climatic data				Dry matter	Analytical data of dry matter					
	Tem- per- ature	Tem- per- ature coeff- icient	Radi- ation calor- ies per square centi- meter	Vapor pres- sure deficit		Insol- uble pro- tein	Sol- uble pro- tein	Rest- soluble N×5	α-Ami- no N	Am- monia N	
	°C			Mm.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
July 31, 6 a. m.-----	21.7	3.30	-----	6.9	11.0	4.3	21.9	2.6	0.20	0.05	
7 p. m.-----	26.7	4.67	570	12.2	12.5	8.1	18.8	3.1	.17	.02	
Aug. 7, 6 a. m.-----	21.1	3.17	-----	6.3	12.8	4.8	17.5	2.2	.16	.04	
7 p. m.-----	23.9	3.85	459	8.4	12.8	4.6	21.3	2.7	.18	.04	
Aug. 14, 6 a. m.-----	16.7	2.33	-----	1.7	10.0	5.2	21.3	2.2	.14	.06	
7 p. m.-----	23.3	3.70	535	9.6	11.3	7.5	19.4	3.6	.09	-----	
Aug. 26, 6 a. m.-----	14.4	2.00	-----	3.4	12.5	7.5	21.3	2.5	.17	.03	
7 p. m.-----	21.1	3.17	590	8.9	13.2	5.0	18.1	2.5	.18	.03	
Sept. 30, 6 a. m.-----	16.1	2.25	-----	5.2	14.8	3.8	19.4	4.5	-----	-----	
Sept. 29, 7 p. m.-----	21.1	3.17	254	8.5	14.5	3.1	17.5	4.5	-----	-----	

Figure 1 is a multi-panel line graph showing physiological data for a cow during a 16-day period in August. The x-axis represents time in hours (2 p.m. to 6 a.m.) and days (7 to 18). The y-axes represent various physiological and environmental metrics.

Top Panel (Metabolic and Environmental Data):

- CALORIES PER SQ. CM.**: Scale 0 to 500.
- TEMPERATURE EFFICIENCY RADIATION**: Scale 0 to 4.
- TEMPERATURE °C.**: Scale 10 to 30.
- SATURATION DEFICIT**: Scale 0 to 15.

Bottom Panel (Nutrient Concentrations in PER CENT):

- DRY MATTER**: Scale 0 to 15.
- SOLUBLE PROTEIN**: Scale 0 to 15.
- INSOLUBLE PROTEIN**: Scale 0 to 15.
- REST-SOLUBLE N**: Scale 0 to 15.
- GLUCOSE**: Scale 0 to 15.
- SUCROSE**: Scale 0 to 15.

The graph shows significant fluctuations in all parameters over the 16-day period, with notable peaks and troughs in temperature, radiation, and nutrient concentrations.

RESULTS OF 1923

In 1923 the services of several workers were available. Even with this unusual amount of assistance it was necessary to abandon detailed separation of the nitrogenous constituents in order to maintain a schedule of two or more samplings per day. More importance was attached, however, to the uninterrupted daily samplings and analyses of petioles and roots, in addition to leaf blades. It appears, also, that it was possible to select more favorable hours of the day than in previous seasons for obtaining marked and significant differences of composition. The data are presented in Tables 5 to 8, and Figures 3 to 8, inclusive.

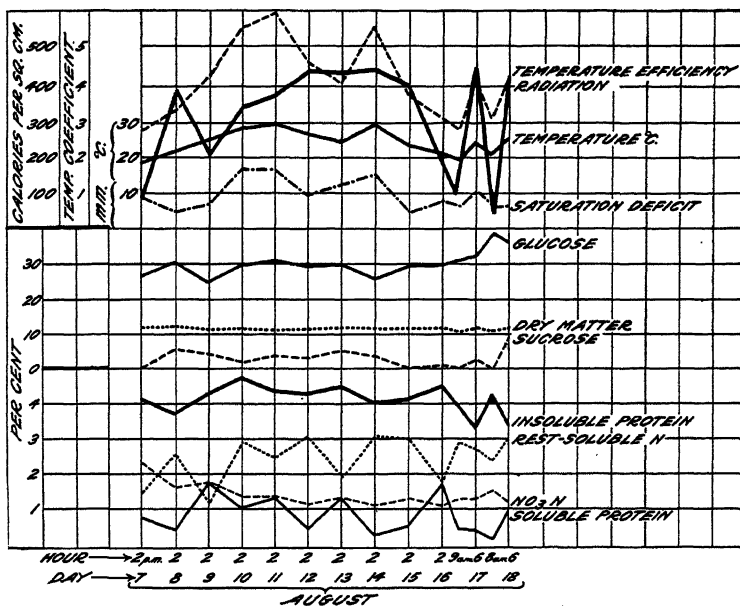


Fig. 4.—Graphs of climatic and analytical data of 1923 investigations of the petiole of sugar beet

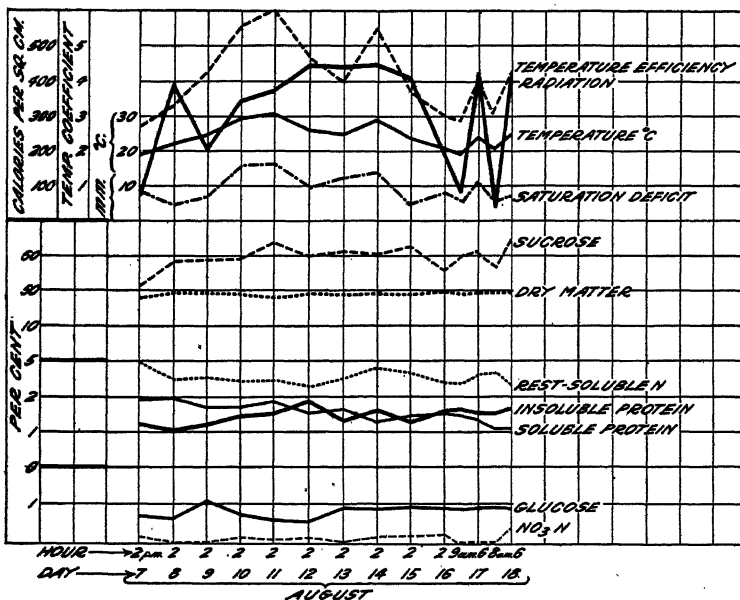


Fig. 5.—Graphs of climatic and analytical data of 1923 investigations of the root of sugar beet

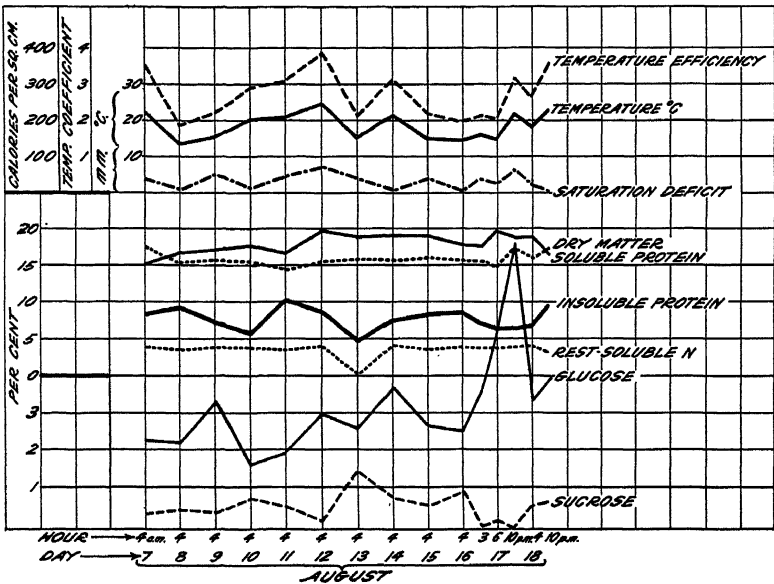


FIG. 6.—Graphs of climatic and analytical data of 1923 investigations of the leaf of sugar beet

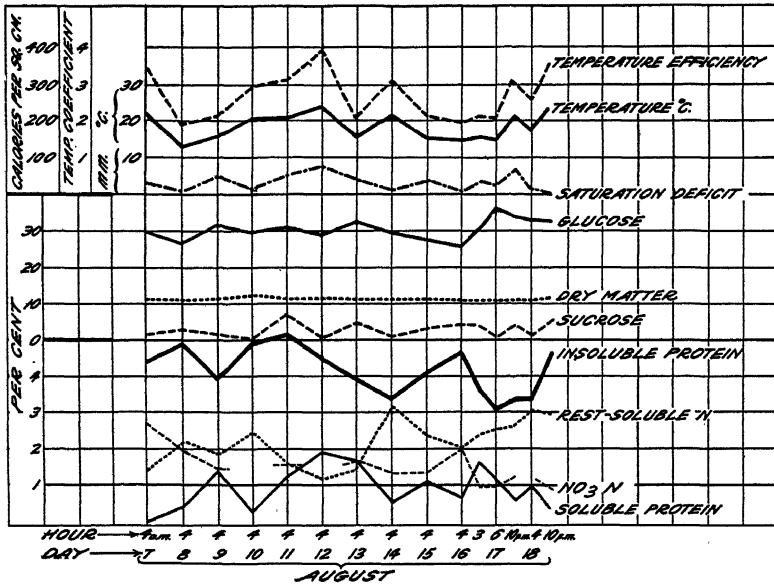


FIG. 7.—Graphs of climatic and analytical data of 1923 investigations of the petiole of sugar beet

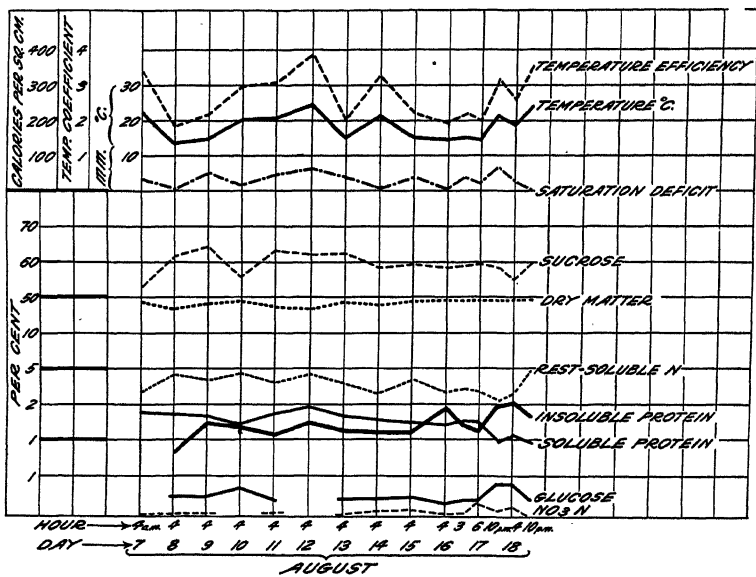


FIG. 8.—Graphs of climatic and analytical data of 1923 investigations of the roots of sugar beet

TABLE 5.—Climatic data of sugar-beet sampling, 1923 (figs. 3 to 8)

Date	Tem- per- ature	Tem- per- ature effi- ciency index	Vapor pres- sure deficit	Radia- tion, calories per square centi- meter ¹	Date	Tem- per- ature	Tem- per- ature effi- ciency index	Vapor pres- sure deficit	Radia- tion, cal- ories per square centi- meter
	° C.		Mm.			° C.		Mm.	
Aug. 7, 4 a.m.	22.8	3.6	2.9		Aug. 15, 4 a.m.	15.6	2.2	3.6	
2 p.m.	18.9	2.7	8.6	73	2 p.m.	23.3	3.7	4.5	403
Aug. 8, 4 a.m.	13.3	1.9	.9		Aug. 16, 4 a.m.	13.9	1.9	.5	
2 p.m.	22.2	3.4	4.4	397	2 p.m.	20.6	3.1	8.5	207
Aug. 9, 4 a.m.	15.6	2.2	5.3		Aug. 17, 3 a.m.	15.6	2.2	3.2	
2 p.m.	25.6	4.3	7.4	209	6 a.m.	14.4	2.0	2.3	
Aug. 10, 4 a.m.	20.0	2.9	1.7		9 a.m.	19.4	2.8	5.7	90
2 p.m.	29.4	5.7	16.8	339	6 p.m.	25.0	4.2	10.3	435
Aug. 11, 4 a.m.	20.6	3.1	5.2		10 p.m.	21.1	3.2	6.9	
2 p.m.	30.6	6.1	17.3	373	Aug. 18, 4 a.m.	18.3	2.6	2.0	
Aug. 12, 4 a.m.	23.9	3.9	7.1		8 a.m.	20.6	3.1	5.4	40
2 p.m.	26.7	4.7	9.4	450	6 p.m.	25.6	4.3	6.8	411
Aug. 13, 4 a.m.	15.0	2.1	3.8		10 p.m.	22.8	3.6	.0	
2 p.m.	24.4	4.0	12.3	434					
Aug. 14, 4 a.m.	21.1	3.2	.9						
2 p.m.	29.4	5.7	14.3	447					

sugar mangold (6), and those of Davis, Daish, and Sawyer obtained in the warm weather of August (9). The latter, however, found up to 12 per cent of hexoses in the cool weather of October.

Sucrose varied irregularly. Over a considerable portion of the period investigated it followed the course of reducing sugars, but at other times, especially from August 9 to 12, it varied inversely as the latter sugar. In view of these results it appears that glucose, as the preponderant form of reducing sugars, fluctuates more like a primary product of carbon assimilation than does sucrose.

The soluble protein in the leaf blades varied distinctly in a direction inverse to temperature. This is in agreement with the results of the previous seasons. Insoluble protein varied irregularly, but, with the exception of the period August 14 to 17, it fluctuated parallel to temperature, and hence inversely to soluble protein. This suggests a relation logically to be expected—namely, inter-conversion between soluble and insoluble protein. It appears significant, also, that insoluble protein varies inversely as reducing sugars, suggesting the possibility of the sequence: Hexose—soluble protein—insoluble protein. The relatively slight variations of rest-soluble nitrogen are also in the direction of those for reducing sugars, suggesting a direct relation of such carbohydrates to synthesis of these forms of nitrogenous compounds.

As the dry matter is relatively constant and shows no pronounced effects of saturation deficit of the air, it may be concluded that the latter factor had little influence in producing the effects analyzed here.

EFFECTS IN THE PETIOLE

In the petiole the variations of dry matter are insignificant, the effects of the saturation deficit of the air appearing to be negligible. The percentage of reducing sugars shows relatively little variation, but there is a distant correspondence to variations of reducing sugars in the leaf. It is partially related in the inverse direction to temperature changes. The small amounts of sucrose vary roughly in the same direction as reducing sugars. They therefore correlate with the monosaccharides of the leaf and not with its sucrose content. It appears from the data that invert sugar is the migratory form of carbohydrates, as generally observed by others.

The soluble protein in the petiole varies irregularly, but seems to be inversely related to radiation. Insoluble protein is generally variable with the soluble form. The rest-soluble nitrogen is distinctly related inversely to soluble protein. It appears to be positively related to temperature, hence varying inversely to the fluctuations of reducing sugars. Nitrate shows an inverse relation to reducing sugars and to rest-soluble nitrogen, while largely parallel to soluble protein. It thus appears that NO_3 is transformed to rest-soluble forms of nitrogen as hexoses accumulate, but that the soluble and insoluble proteins diminish simultaneously.

EFFECTS IN THE ROOT

Variations of the dry matter in the root are negligible and incapable of modifying metabolism. The percentage of reducing sugars varies appreciably in the forepart of the period of observation, as if depressed by rising temperature, but it is almost constant later, despite sharp variations of both temperature and illumination. Sucrose shows a distinct correlation with radiation. As

might be expected in a storage organ highly charged with this reserve, the variations were proportionately much less than those of the leaf. This constituent seems to follow primarily the variations of reducing sugars of both leaf and petiole, rather than those of sucrose therein.

Soluble protein shows here a response generally inverse to temperature changes, while insoluble protein varied relatively directly with this factor. Where there are disagreements, as on August 11 to 13, the response seems to be delayed. The rest-soluble nitrogen varies irregularly. Aside from August 14 to 17, it appears to be inversely related to temperature, and hence correlated with the formation of soluble protein. The variations of nitrate are suggestive of an inverse relation to reducing sugars in the fore period and to rest-soluble nitrogen later. However, the amount is too small to be interpreted as being of significance, especially in view of the possible complication from equilibrium in the root between nitrate diffusing backward from the petiole and that undergoing translocation from the soil.

EFFECTS IN DARKNESS

EFFECTS IN THE LEAF

With the exception of August 9 and the afternoon hours of August 17, the percentage of reducing sugars in the leaf varies with temperature. The marked aberrations of August 9 and excessive effect of the evening of August 17 may well be due to the high illumination of the preceding daylight period. Sucrose varies consistently in a manner inverse to temperature, and hence inversely to reducing sugars. Variations of soluble protein show a tendency to follow temperature at the fore period of growth, but are not distinctly correlated therewith. With the exception of August 13 and 14, insoluble protein varies in a manner inverse to temperature changes. Thus, the proteins of the leaf tend to show by night a temperature response inverse to that of the daytime. The rest-soluble nitrogen shows only one distinct variation (on August 13), and that is parallel to the changes of temperature.

EFFECTS IN THE PETIOLE

The reducing sugars in the petiole vary irregularly with reference to temperature. Sucrose behaves similarly. Excepting the dates from August 11 to 13, soluble protein varied in an inverse relation to temperature. Insoluble protein also varied chiefly in the above manner. Thus these compounds show the same trend in the petiole as by day. The rest-soluble nitrogen varies quite irregularly. Nitrate trends toward an inverse relation to temperature. It therefore shows a rather distinct inverse relation to rest-soluble nitrogen and to reducing sugars.

EFFECTS IN THE ROOT

The percentage of reducing sugars in the root shows no correlation with climatic factors. Sucrose varies in a manner inverse to temperature changes. The latter sugar, therefore, follows its own variations in the leaf. Soluble protein shows a somewhat inverse relation to temperature, and the same is true of insoluble protein. Rest-soluble nitrogen is also irregular, but with a tendency to follow the course of temperature. The slight amounts and variations of nitrate can not be considered significant.

Of the preceding particulars, the following relations appear to be most definite: During exposure to daylight the reducing sugars increase in the leaf with illumination, when the temperature remains well below 30° C. Rest-soluble nitrogen varies parallel to reducing sugars. The relative amounts of these various compounds suggest the sequence: Reducing sugars—amino acids—soluble proteins—insoluble proteins. Simultaneously, the sugars of the petiole are determined somewhat by the variations of reducing sugar in the leaf, but the proteins show little correlation with climatic factors. The variations of nitrates and rest-soluble nitrogen are such as to indicate the sequence: Reducing sugars—rest-soluble nitrogen. Sucrose in the root responds to the variations of reducing sugars in leaf and petiole. Protein is stored in insoluble form at higher temperatures. In general, the results show the delayed response to be expected between leaf and root.

In darkness, the reducing sugars of the leaf increase at the expense of sucrose as the temperature rises. Simultaneously, the proteins also undergo hydrolysis and migrate to the petiole. In the latter organ the same status of proteins is maintained, indicating further migration of these compounds to the root at higher temperatures. The variations of nitrate in the petiole suggest that these, in conjunction with reducing sugars, are converted to rest-soluble forms, although this synthetic process may occur in the leaf blade. Sucrose in the root shows an inverse response to temperature during darkness, but proteins are less definitely influenced.

DISCUSSION

Since the early investigations of Brown and Morris, the nature of the first sugar produced in carbon assimilation of chlorophyllous plants has been a mooted question. These workers worked with leaves of *nasturtium* dried in a steam-heated oven. Fortunately, this tissue dries rapidly, so that the chance of caramelizing is relatively low. The analytical methods employed were the most reliable then extant. As a result of marked accumulation of sucrose during illumination by sunlight, while the reducing sugars varied irregularly, Brown and Morris (2) inclined to the belief that the former was the first sugar of photosynthesis. Parkin (15) followed the distribution of sugars in the leaf of the snowdrop (*Galanthus nivalis*). On exposure of etiolated plants and of detached leaves to daylight, sucrose increased very decidedly while the percentage of hexoses remained rather constant. The ratio of sucrose to hexoses decreased from the tip of the leaves toward the base, and increased as the season advanced. Parkin suggests it is quite possible that sucrose is the first tangible product of carbon assimilation.

Strakosch (21) observed, by means of the osazone reaction, that glucose seemed to be the first sugar formed in the mesophyll of the sugar-beet leaf. The reduction test with Fehling's solution upon tissue extracts showed a great preponderance of hexoses over sucrose in the leaf. Upon exposure to sunlight after prolonged etiolation the hexoses of the leaf were rapidly converted to sucrose. Similar observations led Dixon and Mason (10) to suggest that hexoses are synthesized in the protoplasm, sucrose being condensed therefrom and excreted into the vacuoles.

Campbell (6), on the basis of reliable methods of extraction and analysis, but with admittedly too few data to be conclusive, obtained

evidence that an increase of hexoses precedes that of sucrose in leaves of the sugar mangold with the advent of daylight periods.

Davis, Daish, and Sawyer (9) present data for the distribution of carbohydrates in leaves of the mangold at intervals of two hours. These cover three days from August 26 to October 11. The variations of sucrose exceed those of the hexoses. On account of the low temperatures prevailing on their later dates of examination, their data (Table 9) are not comparable with those of the present writers. The results of August 26-27 are shown graphically in

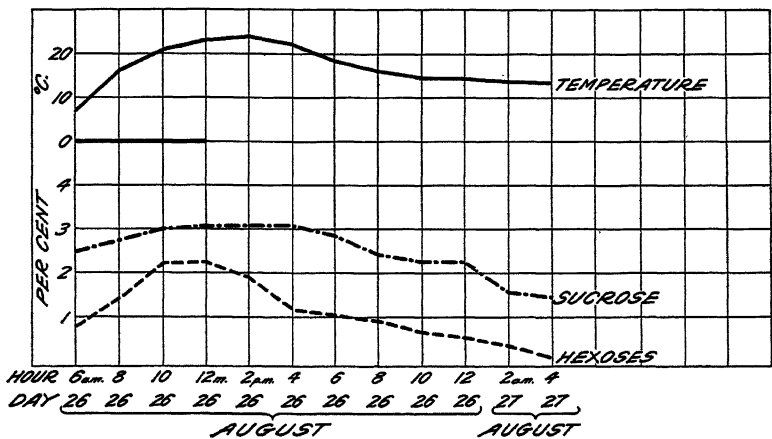


FIG. 9.—Graphs illustrating data of Davis, Daish, and Sawyer on distribution of carbohydrates in leaf of the mangold at intervals of two hours, Aug. 26-27

Figure 9. The percentage of hexoses declined after 10 a. m., when the temperature had reached 21°C. The total sugars also attained a maximum value by 10 a. m., and this plane was maintained until toward 2 p. m. Because of the generally greater variation of hexoses than of sucrose, these investigators consider that their results support the conception of the latter sugar as the primary one of carbon assimilation. As to the relative response of the sugars to the change from darkness to daylight, their results are inconclusive.

TABLE 9.—Data from Davis, Daish, and Sawyer for Figure 9

Date	Temper- ature	Hexose	Sucrose	Date	Temper- ature	Hexose	Sucrose
	° C.	Per cent	Per cent		° C.	Per cent	Per cent
Aug. 26, 6 a. m.	7.2	0.8	2.5	Aug. 26, 6 p. m.	18.9	1.0	2.8
8 a. m.	16.8	1.4	2.8	8 p. m.	16.1	.9	2.4
10 a. m.	21.1	2.2	3.0	10 p. m.	14.7	.9	2.2
12 a. m.	22.9	2.2	3.1	12 p. m.	14.4	.6	2.2
2 p. m.	23.9	1.9	3.1	Aug. 27, 2 a. m.	13.9	.4	1.6
4 p. m.	21.7	1.2	3.1	4 a. m.	13.3	.2	1.5

The accumulation of sucrose while hexoses are decreasing possibly may be explained by the respiratory effect of relatively high temperatures. Brown and Morris (2) estimated that glucose was utilized to a greater extent than fructose in respiration. Lindet (12) grew the embryo of barley on invert sugar, and found a utilization of 17 to 70 per cent more glucose than fructose. If this is true generally,

one can understand what prompted Boysen-Jensen (1) to make his observation on the accumulation of sucrose in germinating peas. If fructose accumulates, even though the plane of glucose is reduced, it would seem quite possible for the condition of equilibrium between these sugars to induce the formation of sucrose.

Thoday (23) quotes Brooks' results from weighings of leaf sections which indicate the cessation of photosynthesis at midday. Thoday ascribed this to engorgement of the leaf tissue with photosynthetic products. It would seem, recognizing limitations of Brooks' method of measurement and his unknown temperature relations, that the effect here discussed might have been due to an excess effect of respiration over synthesis. Spoehr (20, *pt. 2*) has shown the interrelation between respiration and photosynthesis of carbohydrates in leaves of the bean and sunflower. He found that an increase of photosynthesis definitely stimulated respiration. It appears that this effect must be taken into account in interpreting the results of others cited here and those of the present writers which indicate that high temperatures cause a depression in the percentage of reducing sugars in leaf-blade tissue. Moreover, if one considers the criticism of Brown and Heise (3, 4), the rate of carbon assimilation is not augmented much by an increase of temperature and it decreases with increasing illumination. Thus, in the writers' results for 1923 the overstepping of the most efficient light intensity may have operated in conjunction with higher temperatures to produce the limited content of reducing sugar attained. The writers interpret their results as indicating that these sugars are among the primary products appearing in carbon assimilation.

The writers recognize the claim of Chibnall (7) that computations based upon fresh weight of tissue overcome errors incident to determinations based upon dry weight. Nevertheless, they are unable to comprehend how the former method of calculation could alter the proportions of constituents when the moisture content fluctuates little. It is likewise difficult to conceive how allowance for large variations in moisture content could compensate differences in the rate of either synthesis or translocation of constituents in the leaf dry matter. Even the method of determining diurnal changes in tissue components by computation of absolute amounts per individual leaf can not be considered reliable, because of the possible removal or alteration of compounds contemporaneously with synthesis of the same. The latter factors might be compensated in part by including analyses of the other organs of the plant with those of the leaf. When computed to the basis of fresh weight of tissue, the writers' data of Table 6 show a corresponding increase of sugars in the afternoon. None of the relative diurnal values for other constituents are altered, except in the case of soluble protein, in which case the percentage is higher in the afternoon than in the morning on 9 of the 12 dates. As shown by their preceding statements, however, the writers do not consider this relation final proof of maximum synthesis of protein in daylight. In view of the limitations imposed upon separation of products of catabolism from those of anabolism, it seems necessary to consider simultaneous changes of composition in different organs of the plant. This situation greatly complicates the problem considered here. The writers have attempted to deal with it in the preceding survey of their results.

The results reported here give promise that it will be possible to determine the effects of specific climatic factors upon metabolism by means of chemical analysis of the plant tissue at frequent intervals, accumulating sufficient data to justify the computation of correlation coefficients. Perhaps conclusive results can be obtained in this way as surely as by the use of special equipment for the control of environmental factors, more particularly of temperature, atmospheric humidity, and illumination. The capacity of this type of equipment for plant yields must remain quite limited, except at exorbitant cost. Even when results have been tested under such artificial conditions, there remains the problem of determining to what extent the effects discovered obtain under the climatic complex to which field practice is subject.

SUMMARY

Results are presented for diurnal changes of chemical composition in leaf blades of the sugar mangold and sugar beet, and in the petiole and root of the latter plant.

The percentage of reducing sugars in the leaf blade increases with solar radiation, within limits. Temperature seems to be a limiting factor in the increase of these sugars when its value approaches 30° C.

The percentage of soluble protein in the leaf varies in a manner inversely related to temperature and therefore correlated with the fluctuation of reducing sugars. This constituent gave a correlation with temperature of -0.390 ± 0.106 for periods of light and darkness combined.

The foliar fluctuations of reducing sugars are distantly paralleled by the deposition of sucrose in the root, while relatively high temperatures increase the percentage of protein stored in this organ.

The relations in the petiole are such as to suggest that a high plane of reducing sugars in the plant, together with the presence of nitrates, leads to the formation of amino acids and rest-soluble nitrogen.

These results explain practical observations that cool, fair weather, such as that common to the autumn season, is favorable to the storing of high percentages of sugar in the root of the sugar beet.

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DECOMPOSITION OF ORGANIC MATTER IN SOIL ¹

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INTRODUCTION

The chemical and biological decomposition of organic matter in the form of green manure when incorporated with the soil is of fundamental importance in improving soil conditions and plant growth. Green manuring is an important means of restoring soil fertility, and is especially applicable to Virginia agriculture, as most of the soil types in Virginia are very deficient in organic matter. This subject is of great economic importance, and the writer believes that further investigation of the effects of green manures on soils is needed.

Former work (3) ² on this subject at the Virginia Agricultural Experiment Station has shown that when such crops as blue grass, soy beans, rye straw, and clover are incorporated with the soil their organic nitrogen rapidly passes over into nitrates, and that the bacterial flora of the soil is improved by such treatment. In addition to these facts it has been shown by experiment that plant growth has been materially stimulated by the addition of certain green materials to the soil.

Another phase of green manuring that has received attention at this station is the so-called acidity resulting from the turning under of green crops. Results showed that the general belief among farmers that decomposing organic matter in the soil produced acid conditions which are injurious to the growth of crops was not warranted. There was a slight acidity in the early stages of growth, but the acidity resulting from the turning under of the very tender grasses was not permanent. The general tendency was toward the creation of an alkaline state rather than an acid one, and if proper consideration is given to fertilizer treatment which contains a place for lime it seems that no harm results from green manuring.

The phase of the subject with which the subsequent experiments are concerned takes into consideration the stage of growth of the green manure crop in relation to its effectiveness when incorporated with the soil.

Rye is a much used green-manure crop. This plant varies widely in chemical composition between April 15 and June 15. Not only does the composition vary, but succulency diminishes as growth advances, and one would expect the rate of decomposition in the soil to be slower the nearer the crop is to maturity when turned under.

In studying the chemical composition of green-manure crops through successive stages of their growth, it will be seen that as the

¹ Received for publication Dec. 17, 1925; issued July, 1926.

² Reference is made by number (italics) to "Literature cited," p. 98.

nitrogen of the plant diminishes the crude fiber increases, therefore one would expect the younger cuttings to contain the relatively greater amount of nitrogen and the lesser amount of crude fiber. As this gap between the nitrogen and crude fiber widens, there is reason to believe that the change will have its effect on the rate of decomposition when the green manure is turned into the soil.

In former experiments (3) it has been proved that cellulose or woody fiber, in a pure state, decomposes very slowly in the soil. If green-manure crops may be likened to impure forms of cellulose, enriched by various substances, a study of the pure and impure forms should throw some light on the very important practice of green manuring.

It is hoped that the results presented here will give some idea as to the best time to turn under green crops so that they will have the maximum value in replenishing the soil's store of organic matter.

REVIEW OF PREVIOUS INVESTIGATIONS

It appears from the literature that scientific effort has been largely directed toward the chemical rather than the bacteriological side of the question. The bacterial life of the soil plays an important part in breaking up the complex organic compounds, in the formation of humus, and in the formation of nitrates so necessary for the plant's development.

The published work on green manuring has been of a very general nature, and very few investigators seem to have attempted to establish the exact time at which green organic matter should be turned into the soil to give the greatest aid to the many complex activities of the soil flora.

White (12), experimenting with clover as a green manure, found that the younger the crop when turned under the more rapidly it decomposed. He also found that there was a stimulating effect on a later tomato crop.

Hutchinson and Milligan (4) measured the decay of organic matter in the soil by the rate of nitrate accumulation. These investigators observed that nitrification diminished with the age of the green manures applied.

Baessler (2), investigating the value of paulines, serradella, crimson clover, and hairy vetch as green manure plants, in humus sandy soils, advised turning under as late as possible in the life of the plants, and not to turn under in the hot summer when the plants are green.

Pfeiffer (8, p. 13), in his studies on the nitrogen assimilating bacteria, found that the open sandy soils gave the best results when green manures were applied. The same investigator and three of his associates (7, p. 733) noted a harmful effect when straw was used as a green-manure crop.

Maynard (5), using the accumulation of nitrates as an indication of the rate of decomposition of sweet clover, found that the rate of decay of the sweet clover decreased as the plant became more mature.

Merkle (6), investigating the decomposition of organic matter in soils, used as a measure of decay the formation of humus and the evolution of carbon dioxide, and found that the more succulent the plant the more rapidly does it decay in the soil.

Starkey (10) found that the decomposition of organic matter in the soil was closely related to the evolved carbon dioxide, and that the evolution of this gas served as an index to the processes of decomposition. He experimented with cellulose, rye straw, alfalfa meal, fungous material, dried blood, and dextrose.

Waksman and Heukelekian (11) found that the determination of the power of a soil to decompose cellulose can be added to the group of methods used for carrying out a microbiological analysis of a soil. They suggested certain methods to measure the cellulose-decomposing power of the soil, each method yielding information viewed from a different angle.

Seelhorst (9) observed that when green manures are turned under in the fall more nitrogen is lost and less is found in the succeeding crop. This investigator found that the late turning under of green manure had many disadvantages, and in every case the nitrogen in the crop was greater with spring green manuring.

Whiting and Schoonover (13), in greenhouse and field experiments, compared the rate of decomposition of green and cured clover tops in soil, and found that the process of curing retarded the rate of decomposition, as measured by ammonification, nitrification, and loss of carbon. In commenting on the hypothetical reasons for the differences in rate of decomposition between green and cured clover, they say:

In considering the greater initial rate of decomposition exhibited by the green clover as found by all methods used to study the decomposition, it did not appear that there were any indications of purely chemical changes having occurred in the clover during dehydration. Enzymic action as a factor in affecting the cell contents is not an impossibility, but the temperature at which the green clover was held during the dehydration of the lot with which it was compared, would exclude the possibility of much change in it, while the dehydration process with the other lot of clover would require the action of the synthetic enzymic processes to retard its rate of decomposition by stabilizing the nitrogen compounds.

The explanation that seems to fit the results found is based on a physical hypothesis. The loss of water is of course accompanied with an increase in the concentration of salts in the cells. This brings about a hardening of the cell contents, and the whole material becomes more horny and shriveled. The diffusion of nitrogenous substances is stopped until hydration has been reestablished. The colloidal nature of the cell contents causes them to become hydrated slowly, after a previous dehydration. Apparently the time required to bring about the hydration in a normal soil is sufficiently long for the ammonifying bacteria to gain headway on the green material, and consequently to effect a much greater rate of decomposition than those acting on the dehydrated material.

In a previous work (3) the effect of cellulose was studied with respect to nitrification in several leading types of Virginia soil originating from distinct geological formations. The results showed a depression of nitrate in every case, and in many instances the nitrate already present in the soil was used up. There was a decided diminution in plant growth, with a yellow appearance in all the plants grown in a cellulose-containing medium. The following work was carried out with the object of determining the effect of cellulose on the plant when the nitrogen supply was in a very soluble form.

EFFECT OF CELLULOSE ON PLANT GROWTH IN THE PRESENCE OF NITROGEN IN A VERY SOLUBLE FORM

The soil selected for this work represented a typical Hagerstown silt loam, and came from an unfertilized plot that had been in corn for about eight years. The soil was practically exhausted as far

as nitrate nitrogen was concerned, for on analysis it showed only 0.6 milligram of nitrogen in each 100 grams of soil. A soil containing such a small amount of this substance is an ideal medium for study when enriched with organic matter. The pots used were of the Wagner pattern, with side tubes, and of 12-kilogram capacity. Corn was the crop used as the indicator.

In previous work on nitrate formation where green crops had been turned under, the calculations for the additions of cellulose were made on a 4-ton basis, or 35.5 grams per pot of 12-kilogram capacity. Taking these figures as a basis, the amount of cellulose was varied accordingly, beginning with an addition of cellulose representing 1 ton and increasing the amount to about 28 tons per acre.

In the first series of experiments the quantities of cellulose added to the soil varied, but no nitrate was added. The second series contained soils varying in cellulose, with the nitrate constant. A third series was run, varying both cellulose and nitrate. Where the nitrate was constant, one-hundredth of 1 per cent of potassium nitrate was added to the mixtures.

TABLE 1.—*Effect of cellulose on the growth of corn in pots, varying amounts of cellulose being added to Hagerstown silt loam soil but without the addition of any fertilizer materials*

Pot No.	Cellulose applied	Weight of plants produced		Pot No.	Cellulose applied	Weight of plants produced	
		Green	Dry			Green	Dry
	Gm.	Gm.	Gm.		Gm.	Gm.	Gm.
1.....	8.87	210.0	52.3	6.....	142.00	4.6	1.9
2.....	17.75	150.4	30.5	7.....	177.50	5.3	1.5
3.....	35.50	102.8	16.7	8.....	213.00	13.1	2.5
4.....	71.00	162.9	25.8	9.....	248.50	2.7	1.4
5.....	106.50	23.7	5.2				

It will be seen from Table 1 that apparently no marked injurious effect was shown by the addition of cellulose to the soil until quantities exceeding 71 grams (or the equivalent of 8 tons of organic matter per acre) had been reached. Above this amount the growth of the plants was restricted to a marked degree. In pot 9, receiving 248.50 grams of organic matter, plant growth almost stopped.

TABLE 2.—*Effect of cellulose and potassium nitrate on the growth of corn in pots, varying amounts of cellulose and a constant amount of potassium nitrate being added to Hagerstown silt loam*

Pot No.	Treatment		Weight of plants produced		Pot No.	Treatment		Weight of plants produced	
	Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry		Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry
	Gm.	Per cent	Gm.	Gm.		Gm.	Per cent	Gm.	Gm.
10.....	8.87	0.01	250.7	49.9	15.....	142.00	0.01	2.3	1.0
11.....	17.75	.01	142.4	21.2	16.....	177.50	.01	3.0	1.0
12.....	35.50	.01	86.4	15.0	17.....	213.00	.01	3.5	1.3
13.....	71.00	.01	27.3	3.4	18.....	248.50	.01	5.8	2.3
14.....	106.50	.01	7.7	1.4					

A constant quantity of nitrate failed to counteract the injurious effects of increasing quantities of cellulose added to the soil. The results in Table 2 duplicate those shown in Table 1 fairly closely. There is a gradual restriction of plant growth in all pots above the 8-ton-per-acre application, and the addition of 0.01 per cent potassium nitrate failed to counteract the depressing effect of the cellulose.

TABLE 3.—*Effect of cellulose and potassium nitrate on the growth of corn in pots, varying amounts of cellulose and potassium nitrate being added to Hagerstown silt loam*

Pot No.	Treatment		Weight of plants produced		Pot No.	Treatment		Weight of plants produced	
	Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry		Cellulose applied to each pot	Potassium nitrate applied to each pot	Green	Dry
	Gm.	Per cent	Gm.	Gm.		Gm.	Per cent	Gm.	Gm.
19.....	8.87	0.001	341.3	42.8	25.....	177.50	0.100	143.3	17.5
20.....	17.75	.003	366.1	38.5	26.....	213.00	.300	13.1	3.1
21.....	35.50	.008	256.2	37.7	27.....	248.50	.500	7.6	2.7
22.....	71.00	.009	330.0	33.5	28.....	Check.		283.2	63.6
23.....	106.50	.010	363.0	37.0	29.....	Check.		265.5	63.8
24.....	142.00	.050	295.3	33.5					

Varying the quantity of nitrate had very little effect on plant growth in the above series, except that the stimulating effect of the nitrate was carried a little further than in the former experiments. Plant growth in the former studies was diminished after 8 tons per acre of the organic matter had been added, but increasing the amount of nitrate tended to increase plant growth slightly beyond this stage. It is evident that keeping such apparently inert material as cellulose at about 1 ton per acre, and increasing the quantity of nitrate, will eliminate many of the ill effects of this substance. It is apparent in applying these results to substances containing a wide nitrogen-carbon ratio, such as mature rye and wheat straw, that additions of nitrate will hasten their decomposition and availability to the plant.

A striking fact is shown in Table 3 concerning the weight of the plants. Regardless of the quantities of potassium nitrate added (either the same or smaller or greater than in the preceding experiments) the weight of the plants was uniformly greater in this series than in the other series for corresponding quantities of cellulose applied, except for one green weight and one dry weight. All of the plants were cut at the same time and had reached the same stage of maturity, but the succulency of the crop grown in this experiment (even that part receiving very little nitrate) was greater than in the preceding experiments. This is a very desirable quality in certain crops, especially crops to be turned under as green manures, as subsequent experiments will tend to show that there is a definite stage of growth of green-manure crops which is associated with maximum decomposition, taking into account their chemical composition with reference to nitrogen.

In the above experiment the vigor and health of the plants were much improved over the series which had no nitrate and those receiving the nitrate in constant amount.

EFFECT OF CELLULOSE ON PLANTS GROWN IN A NUTRIENT SOLUTION

The foregoing experiments have shown that there is a diminution in growth and vigor of plants when the amount of the soil-incorporated cellulose is increased, and that the ill effect is proportional to the amount of cellulose added. The addition of potassium nitrate in varying quantities has had little effect in diminishing the effect of cellulose beyond a 4-ton application of the same.

A series of experiments was run to further study this question, cellulose being added to a nutrient solution. This medium was replaced by a fresh solution of the same composition from time to time in some cases, but in others the solution was not renewed and the effect on plant growth was noted.

The nutrient solution selected (Crone's) was of the following composition: Water 1,000 c. c.; potassium nitrate 1 gm.; iron phosphate (ferric) 0.5 gm.; calcium sulphate 0.25 gm.; magnesium sulphate 0.25 gm. It was prepared in 20-liter quantities.

Large stoneware vessels were used for growing the plants, and 35.5 gm. of cellulose was used to 3,000 c. c. of nutrient solution.

As a preliminary study four pots were used, two as checks, in which the nutrient solution was renewed three times each week, and two to which the equivalent of 4 tons of cellulose per acre had been applied and the nutrient solution renewed as in the case of the check pots. Figure 1 shows the effect of the treatments on the plants and their root systems.

Where the cellulose was eliminated the plants were healthy and vigorous and had a rich green color. With the cellulose-treated pots the plants were stunted in growth and had a very yellow, unhealthy appearance, even though the nutrient solution was replenished three times a week. The root systems of the plants receiving no cellulose were healthy and very white and clean, while those of the cellulose-treated pots were dark and gave off a slight odor of hydrogen sulphide. The dry weight of the plants from the check pots averaged 28.7 gm., while the average for the cellulose pots was 4.6 gm. The checks therefore contained more than six times the dry matter as was obtained from the cellulose-treated pots. Nitrogen determinations on these crops were not made, but former experiments (3) have shown a corresponding decrease in nitrogen for plants grown in soils that had been treated with cellulose. These experiments indicate that cellulose restricts plant growth, even though the medium in which the plant is grown is a well-balanced one in every particular.

As a continuation of this study the following series were run, the same nutrient solution being allowed to remain for the whole period in two instances, and it being changed at regular intervals in the others. The plan was as follows:

Pot No.	Nutrient solution	Treatment	Crop results, dry-weight basis
211	No cellulose.....	Renew nutrient solution once each week.....	<i>Gm.</i> 43.6
210	do.....	No renewal of nutrient solution.....	18.4
8	Cellulose.....	Renew nutrient solution twice each week.....	10.7
15	do.....	Renew nutrient solution once each week.....	8.6
16	do.....	No renewal of nutrient solution.....	2.7

In the pots that did not receive the cellulose treatment the plants were vigorous and healthy (fig. 2, A). Changing the solution once each week gave an increase in plant growth amounting to 25.2 gm., dry weight.

When cellulose was added the plants were poor and the root system very much discolored (fig. 2, B). With pot 8, in which the

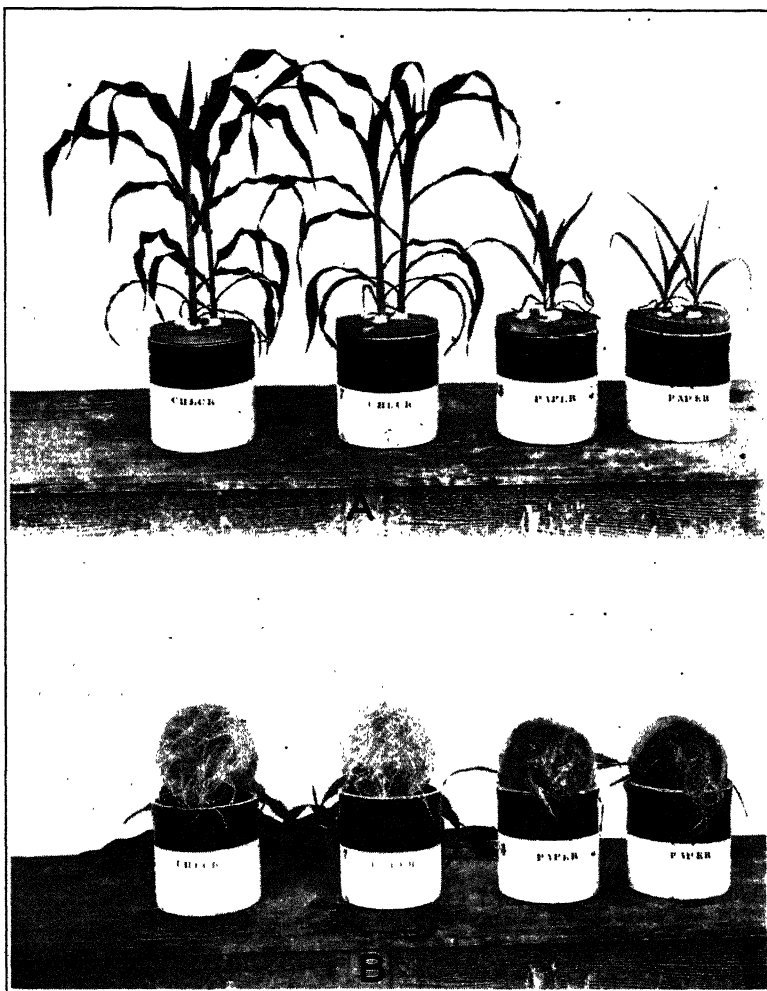


FIG. 1.—A. Showing the effect of cellulose on the growth of corn in pots, using Crone's nutrient solution. B. Root systems of the same plants

nutrient solution was renewed twice a week, there was a slight gain over pot 15, which had the nutrient solution renewed only once each week. Growth was very poor in pot 16, where there was no renewal of the nutrient medium.

There was a copious evolution of hydrogen sulphide in all of the cellulose pots after about four weeks. There was a sufficient quantity of this gas present to give a test with lead acetate. This evolu-

tion of hydrogen sulphide was probably caused by the action of the decomposing organic matter on the sulphates of calcium and magnesium present in the nutrient solution. Diminished growth results.

Apparently the ill effects produced by cellulose were as marked in the nutrient solution as when the plants were grown in soil with the same additions of cellulose.

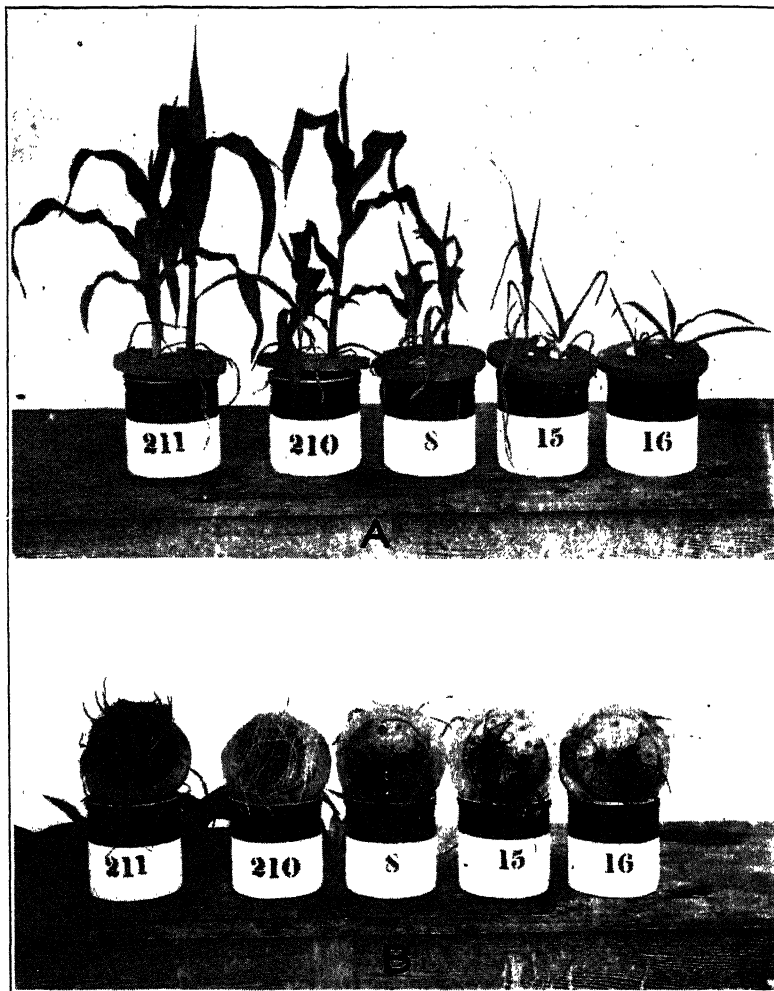


FIG. 2.—A. Showing the effect of cellulose on the growth of corn in pots, when the nutrient solution was renewed at different times. B Root systems of the same plants

Plants contain varying amounts of cellulose, depending more or less upon the other constituents present. In other words, the crude fiber contained in plants may be regarded as a more or less impure form of cellulose, and it is believed that the greater the amount of cellulose contained in the plant the slower will be its decomposition when incorporated with the soil. For instance, it is thought that

young rye cut about April 15 is more readily decomposed in the soil than rye cut on or about June 1, assuming that both were planted at the same time.

Former observations have shown that rye has a very peculiar effect on soil of certain types, there seeming to be a tendency toward restriction of plant growth; however, it is thought that if rye is cut at a certain stage of development some of the objectionable effects may be eliminated.

As a preliminary study of this question the following experiments were undertaken. Large wooden tubs, holding about 75 pounds of soil, were arranged for proper drainage and were kept out of doors. Crimson clover was added to soil and sand at the rate of 5 tons per acre. A typical Hagerstown silt loam from Blacksburg was used in the experiment. Corn was the indicator crop. The average results over a period of three years are given in Table 4.

TABLE 4.—*Effect of crimson clover on the growth of corn in tubs when added to Hagerstown silt loam*

Pot No.	Soil type	Treatment	Dry weight of plants produced
			Gm.
356	Sand.....	Control.....	38.2
357	do.....	do.....	38.7
358	do.....	½ pound crimson clover.....	56.4
359	do.....	do.....	70.6
360	Hagerstown silt loam soil.....	Control.....	61.6
361	do.....	do.....	66.4
362	do.....	½ pound crimson clover.....	87.9
363	do.....	do.....	87.0

The results in Table 4 show that crimson clover has a marked effect on plant growth when turned under in the soil. Even with such inert material as sand the green manure increased plant growth, although the increase over a 3-year period was not so great as with the soil. With sand, there was very little effect the first year, but during the second and third years the effect was very marked. These results were obtained by using one of the best green-manure crops studied both in the greenhouse and in the field. The effect of rye on plant growth is probably not so good, as it is a nonlegume that gives various results, depending upon a number of factors, one of which is soil type, and therefore the following experiments were carried out on four of the leading Virginia soil types. These experiments were conducted in the same way as the preceding ones. (Table 5.)

The results of the experiments shown in Table 5 indicate that rye produced no marked effect in increasing plant growth. On the heavy clay soils of Albemarle and Appomattox there was a depression in plant growth. With the lighter soils of Norfolk and Blacksburg slight gains were observed from the turning under of rye. These experiments were planned as a check on a series of field experiments begun in 1912.

TABLE 5.—*Effect of green rye on different types of Virginia soil*

Plot No.	Soil type	Treatment	Dry weight (grams) 3-year average
298	Albemarle	Control	254.7
299	do.	do.	202.2
300	do.	1 pound green rye	220.5
301	do.	do.	197.6
302	Appomattox Light	Control	226.8
303	do.	do.	242.7
304	do.	1 pound green rye	205.7
305	do.	do.	182.3
306	Blacksburg	Control	250.0
307	do.	do.	233.5
308	do.	1 pound green rye	286.7
309	do.	do.	348.5
310	Norfolk	Control	247.7
311	do.	do.	213.6
312	do.	1 pound green rye	253.0
313	do.	do.	272.3

In the field work, 10 tenth-acre plots made up the series, 5 in corn and 5 in wheat. The green manures used were clover, rye, soy beans, and buckwheat. Results over a period of 12 years have been obtained with corn. The wheat was harvested on alternate years, and yields for six years are available. Tables 6 and 7 give the treatment, gain or loss in nitrogen, and the yields over the period 1912–1923:

TABLE 6.—*Yields of corn, in bushels per acre, for 12 years, under different methods of green manuring; and the effect of this treatment on the total nitrogen of the soil*

Plot No.	Treatment	Per cent nitrogen		Yield, 12-year average
		1912	1923	
1	Clover cut for hay	0.114	0.119	35.18
2	Clover turned under	.118	.125	37.48
3	Check	.076	.064	13.24
4	Rye cut for hay	.144	.090	17.25
5	Rye turned under	.112	.111	8.95

TABLE 7.—*Yields of wheat, in bushels per acre, for 6 years, under different methods of green manuring; and the effect of this treatment on the total nitrogen of the soil*

Plot No.	Treatment	Per cent nitrogen		Yield, 6-year average
		1912	1923	
1	Soy beans cut for hay	0.102	0.079	21.09
2	Soy beans turned under	.074	.123	26.02
3	Check	.074	.089	16.09
4	Buckwheat cut for hay	.090	.091	12.94
5	Buckwheat turned under	.098	.123	17.42

The data in Tables 6 and 7 show that with the possible exception of rye, the green manures have increased the yields of corn and wheat, and at the same time increased the total amount of nitrogen in the soil after giving up that necessary for the production of the crop.

Rye in the field and in greenhouse experiments has exerted a more or less harmful effect on plant growth, with corn as the indicator. This ill effect is apparently more or less temporary, and is probably due to the changes in the first stages of decomposition.

Clover showed a marked beneficial effect on the yield of corn and on the nitrogen reserve of the soil. The physical benefit derived from the turning under of this form of organic matter is very pronounced.

Rye did not increase the nitrogen reserve on plots 4 and 5, and when turned under it depressed the yield. The corn on the check and rye plots had a very yellow appearance, and gave every indication of nitrogen deficiency.

With wheat, green manures gave increases in all cases. This was very marked where soy beans were turned under. The soy-bean stubble did not increase the reserve nitrogen in the soil. The greatest increases in nitrogen were shown where soy beans and buckwheat were turned under, the soy beans giving 980 pounds of nitrogen per acre-foot and the buckwheat 500 pounds.

Lime was not included in the fertilization plan of these experiments, and a substantial addition of it to these plots is planned. It is believed that lime may counteract some of the injurious effects resulting from the incorporation of green rye with the soil. Recent laboratory results indicate that green rye does not nitrify as readily in the green state, but this same rye, when air-dried, gives increased initial nitrification when mixed with the soil. Several kinds of crops used as green manures exhibit this peculiarity. When green manures fresh from the field are mixed with the soil and kept at a definite temperature and with an optimum water content, an initial ammoniacal decomposition results. It may be that this rapid ammoniacal evolution causes the depressing effects often noticed when green manures are first turned under.

DECOMPOSITION OF GREEN MANURES IN THE SOIL, AS MEASURED BY CARBON DIOXIDE EVOLUTION

Analyses made over a period of three years of green manure crops cut and turned under at different periods of development have shown that such crops as rye, oats, clover, and vetch vary materially in composition and in their effects when incorporated with the soil.

At just what period of growth these crops should be turned under to furnish the most desirable form of organic matter is yet to be determined. An elaborate series of plots (80) has been under study at this station for some time, and the results so far obtained indicate that the younger and more succulent plants contain much more nitrogen and less crude fiber, or cellulose, than the more mature plants. The young, succulent plants decompose more rapidly in the soil than crops cut at a more mature stage.

All of the above points indicate that there is an intimate relationship between total carbon and the nitrogen of plants, and that the carbon and nitrogen exert a material effect on decomposition and consequently on the rate of carbon dioxide evolution. That the ratio of these two substances is to be taken into account has been indicated. Bearing these points in mind the following experiments were planned.

Rye, oats, clover, and vetch cut on May 17 when the plants were succulent, vigorous, and healthy, were selected as the forms of organic

matter to be studied. Cellulose in the form of filter paper was included in the series.

The plants were cut and taken to the laboratory, and the moisture was determined immediately. In every instance the equivalent of 0.5 gm. of the material was used, due allowance being made for the water content. The green materials were added to the equivalent of 100 gm. of dry Hagerstown loam soil, and water was added to bring the soils to optimum moisture content. The cellulose was added in like amount.

The soil and organic matter were placed in 500 c. c. Erlenmeyer flasks, and kept in the incubator at 25° C. The carbon dioxide was determined at daily intervals, due consideration being given to the proper flow and purification of the air passing over the mixture of soil and organic matter.

As a basis for future calculations of the rate of carbon dioxide evolution, and the ratio existing between the nitrogen and carbon of the organic matter, the amounts of total nitrogen and total carbon were determined. The methods of the Association of Official Agricultural Chemists were used (1). These data are shown in Table 8.

TABLE 8.—*Nitrogen and carbon in organic matter*

Substance, and date of cutting	Per cent nitrogen	Per cent carbon	Nitrogen-carbon ratio
Cellulose.....		44.44	
Rye, May 17, 1924.....	1.31	38.29	1 to 29.23
Oats, May 17, 1924.....	1.27	38.29	1 to 30.15
Clover, May 17, 1924.....	3.05	38.83	1 to 12.73
Vetch, May 17, 1924.....	3.98	38.92	1 to 9.78

The results given in Table 8 show that the nonlegumes contain much less nitrogen than the legumes, although the materials contained what might be regarded as the maximum amount of nitrogen for each plant at this period of its development. Rye cut at maturity (about June 15 for this locality) contains less than 1 per cent of nitrogen as has been indicated by previous work on the composition of this plant. The same is true of oats. There is a much wider ratio between the nitrogen and carbon of the nonlegumes than of the legumes; therefore one would expect a more rapid decomposition of the latter form of organic matter when mixed with the soil. Table 9 contains data bearing on this point.

In Table 9 no results are given for the soil blanks. These checks ran between 50 and 70 mg. of carbon dioxide for the 10-day period, and in every case the proper deduction was made, therefore the figures given above represent the entire amount of carbon dioxide evolved from the organic matter added in the form of green manure.

The cellulose-treated soil, where no nitrogen was present except that already present in the soil showed the lowest rate of decomposition, only 42.6 mg. of carbon dioxide having been liberated over a 10-day period. For the first three days of the experiment there was practically no decomposition going on. The maximum liberation of carbon dioxide was reached on the fourth day. Decomposition after that day was more or less uniform for the remainder of the experimental period.

TABLE 9.—Decomposition of fresh green materials

DAILY EVOLUTION OF CARBON DIOXIDE (MILLIGRAMS)

Substance	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
Cellulose---	0.8	1.1	1.0	6.7	6.0	5.9	6.0	5.3	5.0	4.8	42.6
Rye-----	29.9	29.3	34.7	57.2	46.8	24.9	22.1	20.0	18.3	17.8	301.0
Oats-----	30.5	28.4	42.7	45.5	30.6	28.3	25.1	24.0	23.2	20.7	299.0
Clover-----	41.7	25.9	34.8	43.9	32.5	32.9	30.0	25.1	22.3	21.6	310.7
Vetch-----	46.4	49.5	65.4	72.0	35.1	39.6	27.4	26.1	25.0	22.1	408.6

DAILY DECOMPOSITION (PER CENT)

Cellulose---	0.98	0.23	0.36	1.18	1.91	2.64	3.37	4.02	4.64	5.22	-----
Rye-----	4.26	8.43	13.37	21.51	28.17	31.72	34.86	37.71	40.31	42.84	-----
Oats-----	4.34	8.38	14.46	20.94	25.30	29.32	32.90	36.32	39.62	42.56	-----
Clover-----	5.85	9.49	14.37	20.53	25.09	29.71	33.92	37.45	40.58	43.60	-----
Vetch-----	6.50	13.43	22.59	32.68	37.59	43.14	46.97	50.63	54.13	57.23	-----

Rye and oats exhibited about the same rate of decomposition. The total for the rye was 301 mg. and for the oats 299 mg. However, there was a slightly greater liberation of carbon dioxide from rye than from oats, as the rye contained slightly more initial nitrogen than the oats. Rye and oats showed a much wider nitrogen-carbon ratio than clover and vetch. Rye and oats reached their maximum decomposition stage on the fourth day, with 57.2 and 45.5 mg. of carbon dioxide, respectively.

Clover and vetch showed greater carbon-dioxide evolution than rye and oats. Vetch gave off much more carbon dioxide than did clover. The maximum decomposition of both clover and vetch was reached on the fourth day. The initial nitrogen in the vetch was 0.93 per cent greater than that in the clover.

With the exception of cellulose, the decomposition of the several forms of organic matter was fairly rapid. The materials used were ground very fine, and all other conditions were favorable to rapid decomposition, probably much more so than would be experienced in the field; but there is every reason to believe that there would be almost as much if not more decomposition under field conditions, as the organic matter would be subjected to a much greater aeration and also to other atmospheric effects which can not be duplicated under artificial conditions. It would therefore appear that these experiments show carbon dioxide liberation closely approaching ordinary field conditions. Observations on clover added to soil in lysimeter rims have shown this material to be entirely converted into humus after a duration of about eight months.

Taking these results as a whole, the different forms of organic matter cut about May 17 give a fairly equal degree of decomposition, with the exception of vetch. Rye, oats, and clover released more than 40 per cent of their carbon dioxide during a 10-day period; with vetch, 57.23 per cent of the total carbon dioxide was evolved.

After the carbon dioxide had been determined in the samples, the mixtures were examined to determine the nature of the fermentation. All of the mixtures, with the exception of the untreated soil, gave a very pungent putrefactive odor, and there were evidences

of a strong ammoniacal fermentation. There was no germination of weed seed in any of the flasks, except the check to which no organic matter had been added, and in this flask the growth was exceptionally abundant.

Nitrate nitrogen determinations were made of the mixtures. The soil gave 2 mgs. of nitrogen as nitrate, and in all of the flasks to which organic matter had been applied not the slightest trace of nitrate nitrogen was found.

EFFECT OF AIR DRYING ON THE DECOMPOSITION OF GREEN MANURES

When the green manures were prepared for the preceding experiments, a certain portion was allowed to dry in air for subsequent study.

It has been thought for some time that there was a difference in the rate of carbon dioxide liberation in green organic matter as compared to the liberation from the same material after it had been thoroughly air dried.

In order to see just what effect air drying has upon green organic matter, a series of flasks was prepared duplicating the former experiments, using air-dried samples of rye, oats, clover, and vetch. The cellulose results of the former experiments were included for comparison. In all cases blanks with soil alone have been deducted from the daily evolution of carbon dioxide. The results are given in Table 10.

TABLE 10.—*Decomposition of materials after air-drying*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Substance	First day	Second day	third day	Fourth day	Fifth day	Sixth day	Seventh day	Elighth day	Ninth day	Tenth day	Total
Cellulose...	0.8	1.1	1.0	6.7	6.0	5.9	6.0	5.3	5.0	4.8	42.6
Rye.....	34.2	46.2	20.2	30.3	17.5	9.3	11.6	4.3	8.0	6.1	187.7
Oats.....	33.2	65.1	28.3	32.8	25.6	11.5	18.0	7.9	11.0	8.7	242.1
Clover.....	47.2	51.6	50.4	40.7	30.8	21.1	18.3	12.6	10.1	8.2	291.0
Vetch.....	39.9	63.9	43.0	64.4	35.9	17.0	20.8	12.3	8.1	7.0	312.3

DAILY DECOMPOSITION (PER CENT)

Cellulose...	0.98	0.23	0.36	1.18	1.91	2.64	3.37	4.02	4.64	5.22	-----
Rye.....	4.87	11.44	14.32	18.63	21.12	22.45	24.10	24.71	25.85	26.72	-----
Oats.....	4.73	13.99	18.02	22.69	26.33	27.97	30.53	31.66	33.22	34.46	-----
Clover.....	6.62	13.87	20.94	26.65	30.98	33.94	36.51	38.27	39.69	40.84	-----
Vetch.....	5.59	14.54	20.56	27.06	32.09	34.47	37.39	39.10	40.24	41.22	-----

The results in Table 10 show that in every case air drying restricted the liberation of carbon dioxide as compared with the liberation from the materials fresh from the field. The loss in milligrams for the different treatments over a 10-day period, as compared with the green additions, was as follows: Rye 113.3; oats 56.9; clover 19.7; and vetch 96.3. Each form of organic matter, except vetch, reached its maximum stage of decomposition on the second day of the experiment. Air drying appeared to affect all treatments materially. This was especially noticeable with the legumes clover and vetch.

The fermentation of the mixtures was of a very different nature from that observed in mixtures of the green organic matter. There was no foul putrefactive odor, and all of the soil treatments were sweet and mellow. In every flask there was an abundant germination of weed seed. This was first observed on the fourth day, and the plants appeared to thrive as the experiment continued.

A chemical examination for nitrates showed their presence in every case, and a quantitative determination gave the following results in milligrams per 100 grams of dry soil: Soil 2; rye 0.3; oats 0.3; clover 1.35; vetch 1. There was a reduction of nitrates when compared with the soil which had received no organic matter. This loss of nitrates was in all probability due to the organism of the soils making use of it as food and as plant food supporting the young plantlets present in the soil.

In accounting for the difference in carbon dioxide liberation between the green and air-dried organic matter, it is thought that certain sugars may have been affected. There is strong probability that the more soluble carbohydrates which would serve directly as nutrients for the biological life necessary to proper decomposition had been changed. The changes in these bodies may have restricted the evolution of carbon dioxide.

In drying the plants it is also possible that the soluble hemicelluloses and other polysaccharides were changed into less soluble forms leading to the development of cellulose or wood fiber.

In an effort to substantiate the above hypotheses, it is planned to study these changes in subsequent experiments, by comparing the analyses of organic matter commonly used as green manures. The green, air-dried, and oven-dried substances will be studied to see if there are changes in the nitrogen-free extract and crude fiber. The different forms of green manure will be extracted with water and alcohol, and the extracts hydrolized with dilute hydrochloric acid, and the resulting invert sugars determined. It is believed that such a method of attack may throw some light on the changes brought about as the result of enzymic action or by the process of drying plant substances.

This work may show why a diminution in the liberation of carbon dioxide was obtained when air-dried forms of organic matter were allowed to decompose in the soil. In addition to these points, some light may be thrown on the subject of green manures being regarded by some as less efficient when turned under in an overripe condition.

EFFECT OF DATE OF CUTTING GREEN MANURES ON THEIR DECOMPOSITION IN THE SOIL

In the field work on green manures at this station, five cutting dates have been selected—April 15, May 1, May 15, June 1, June 15. Samples are not always obtainable on these exact dates, on account of the season, but cuttings made at times approximating these periods have been obtained since the beginning of the experiments. Table 11 shows the stage of development at which the crops were cut.

TABLE 11.—*Stage of development of green-manure crops*

Crop	Date of cutting	Stage of development
Winter rye.....	Apr. 15	6 to 8 inches high; beginning to joint.
Do.....	May 1	12 inches high and well jointed.
Do.....	May 15	Beginning to head.
Do.....	June 1	Milk stage.
Do.....	June 15	Nearly mature.
Winter oats.....	Apr. 15	4 to 6 inches high; beginning to joint.
Do.....	May 1	8 to 10 inches high, and well jointed.
Do.....	May 15	Beginning to head.
Do.....	June 1	Milk stage.
Do.....	June 15	Mature.
Crimson clover.....	Apr. 15	4 to 6 inches high.
Do.....	May 1	12 inches high.
Do.....	May 15	Clover in bloom.
Do.....	June 1	Blossoms beginning to die.
Do.....	June 15	Clover dead ripe.
Winter vetch.....	Apr. 15	8 to 10 inches high.
Do.....	May 1	15 to 18 inches high.
Do.....	May 15	Beginning to bloom.
Do.....	June 1	Vetch in full bloom.
Do.....	June 15	Setting seed.

The stage of development of the crops varied with the season, but the notes given in Table 11 will hold good on an average.

By means of crop yields and other studies, an effort is being made to determine the exact stage of development at which green-manure crops should be turned under to give the best results. For this work cuttings from rye, oats, clover, and vetch have been selected in order that their rate of decomposition might be determined.

Table 12 shows the percentages of nitrogen and carbon in the several forms of organic matter used in the carbon dioxide evolution experiments.

TABLE 12.—*Percentages of nitrogen and carbon in the green-manure crops, and the calculated nitrogen-carbon ratios*

Date of cutting of crop	Nitrogen	Carbon	Nitrogen-carbon ratio
Winter rye:	<i>Per cent</i>	<i>Per cent</i>	
May 14.....	1.21	34.95	1 to 28.88
May 21.....	1.03	35.23	1 to 34.20
May 28.....	.66	37.94	1 to 57.48
June 4.....	.81	39.27	1 to 48.48
June 18.....	.67	38.59	1 to 57.60
Winter oats:			
May 14.....	1.77	37.21	1 to 21.02
May 21.....	2.04	38.77	1 to 19.00
May 28.....	1.44	39.24	1 to 27.25
June 4.....	1.46	39.26	1 to 26.89
June 18.....	1.31	38.90	1 to 29.69
Crimson clover:			
May 14.....	3.09	36.80	1 to 11.91
May 21.....	2.51	43.13	1 to 17.18
May 28.....	1.79	38.33	1 to 21.41
June 4.....	2.12	36.52	1 to 17.23
June 18.....	1.98	38.84	1 to 19.62
Winter vetch:			
May 14.....	4.36	36.37	1 to 8.34
May 21.....	4.46	34.54	1 to 7.74
May 28.....	3.70	37.60	1 to 10.16
June 4.....	2.72	38.89	1 to 14.30
June 18.....	3.72	39.01	1 to 10.49

Table 12 shows that in general the younger cuttings of the crops used as green manures contained the greater percentages of nitrogen. Rye dropped materially in nitrogen composition after May 21, and

showed less than 1 per cent after that time. There was a gradual widening of the nitrogen-carbon ratios, and one would expect the rate of decomposition in the soil to be retarded as the crop approaches maturity. With oats, the first two cuttings gave the higher yields of total nitrogen, but after May 21 the nitrogen was fairly uniform. At maturity the oat crop contained less nitrogen than at any other stage. The legumes clover and vetch gave higher yields of nitrogen on the first two cutting dates. Total nitrogen throughout the five periods was much higher with vetch than clover, and consequently the nitrogen-carbon ratio was much narrower. With such a condition present, the vetch would be expected to decompose much more rapidly in the soil than the clover and thus give off more carbon dioxide.

Table 13 shows that the greatest total decomposition of rye was on the first cutting date. Rye cut June 18 contained about half the relative amount of nitrogen as that cut May 14. While the amount of nitrogen contained in the green manure undoubtedly has a relation to the rapidity of decomposition, the rate of disappearance may not be entirely dependent on the percentage of nitrogen. For instance, the decomposition of the crop cut on May 28 which contained 0.66 per cent nitrogen gave the same amount of carbon dioxide as the cutting of May 21, which contained 1.03 per cent nitrogen. On the next cutting date the amount of nitrogen was found to be 0.81 per cent, with a corresponding rise of 16.1 milligrams of carbon dioxide for the period of the experiment.

Taking the results as a whole, it appears that the greatest rate of decomposition is on or about May 14, and this would probably be the most ideal time for the turning under of this crop in order to get best results.

Comparing rye with other forms of green manure, it appears to decompose more slowly in the soil and therefore shows a much lower rate of carbon-dioxide evolution. In 10 days the youngest cutting gave up 43.54 per cent of its total carbon dioxide, against 27.61 per cent on the cutting of June 18, when the crop was mature.

Nitrate-nitrogen determinations were made of the mixtures after the liberation of the carbon dioxide, and the soil check contained 2.2 mg. of nitrogen as nitrate per 100 gm. of soil. In all of the rye treatments there was a reduction of nitrates, as only traces were found. There was a pronounced germination of weed seed in all of the cultures, and no foul putrefactive fermentation was detected.

TABLE 13.—*Decomposition of rye cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)											
Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	43.8	43.3	50.9	40.4	28.0	21.1	26.2	9.1	8.6	7.9	279.3
May 21.....	42.9	37.9	24.2	38.6	23.1	26.6	15.0	11.0	6.2	5.3	230.8
May 28.....	37.7	45.6	21.3	23.0	27.4	17.6	11.1	22.5	15.2	9.4	230.8
June 4.....	57.4	33.9	28.1	22.8	15.8	30.0	15.0	20.6	14.3	8.2	246.9
June 18.....	29.9	12.4	21.4	27.7	27.2	19.0	26.7	13.2	11.0	7.0	195.5

DAILY DECOMPOSITION (PER CENT)											
Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	6.82	13.58	21.51	27.81	32.17	35.46	39.55	40.97	42.31	43.54	-----
May 21.....	6.64	12.50	16.24	22.21	25.79	29.90	32.22	33.92	34.88	35.70	-----
May 28.....	5.42	11.97	15.03	18.33	22.27	24.80	26.39	29.62	31.81	33.16	-----
June 4.....	7.97	12.67	16.57	19.74	21.93	26.09	28.29	31.15	33.13	34.27	-----
June 18.....	4.22	5.97	9.00	12.91	16.75	19.44	23.21	25.07	26.60	27.61	-----

Table 14 shows that the first three cuttings of oats gave the greatest rate of carbon dioxide liberation over a 10-day period. The amount of carbon dioxide evolved was greater than that from rye with a maximum liberation from the oats cut on May 28. Taking the liberation of carbon dioxide as an index to the rate of decomposition under conditions existing in the field, the third cutting of oats is the best for use as green manures.

The initial nitrogen for oats was greater than that of the rye and as a result there was greater evolution of carbon dioxide.

Soluble nitrogen determination of these samples showed the soil blank to contain 4.8 mg. of nitrate to each 100 gm. of dry soil. With the mixtures the nitrate was present in sufficient quantity to get definite results, although there was a reduction in quantity over the untreated soil. In both cases nonlegumes showed a reduction of the nitrates already present in the soil. Germination was positive, and there was no foul putrefactive odor during the decomposition as was the case when green materials fresh from the field were used.

TABLE 14.—*Decomposition of oats cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	59.9	31.8	73.1	59.1	43.0	16.4	5.2	7.9	3.3	3.2	302.9
May 21.....	115.5	39.0	40.6	32.4	31.0	24.2	11.7	8.8	4.0	2.4	309.6
May 28.....	140.6	56.7	61.6	30.8	15.6	24.6	4.7	3.0	1.8	1.4	340.8
June 4.....	50.7	44.7	64.0	30.0	28.1	26.3	7.4	10.1	6.3	3.8	271.4
June 18.....	42.9	30.8	45.7	28.4	17.6	6.8	14.5	5.4	4.6	3.6	250.3

DAILY DECOMPOSITION (PER CENT)

May 14.....	8.80	20.81	31.54	40.22	46.53	48.94	49.71	50.87	51.35	51.82	-----
May 21.....	16.23	28.74	34.45	39.00	43.36	46.76	48.40	49.64	50.20	50.54	-----
May 28.....	19.53	27.40	35.96	40.24	42.40	45.82	46.47	46.89	47.14	47.33	-----
June 4.....	7.04	13.24	22.12	26.29	30.19	33.84	34.86	34.88	37.14	37.67	-----
June 18.....	6.01	17.32	23.73	27.70	30.17	31.12	33.15	33.91	34.55	35.06	-----

The results in Table 15 show a greater evolution of carbon dioxide from clover than from either of the nonlegumes. This was especially noticeable with the cuttings of May 14 and 21. At this stage of growth the clover contained its maximum nitrogen. The greatest evolution of carbon dioxide was in the earlier days of the experiment, and it appears from these results that the young grass is decidedly the best for use as a green manure crop.

Residual nitrates were in greater quantity than in the case of the nonlegumes. The soil blank contained 0.13 mg. of nitrate nitrogen for each 100 gm. of dry soil, and there was an increase in the treated pots throughout the entire period of the experiment, the maximum nitrification being obtained with clover cut on May 21.

Germination of native seed in the soil was abundant in all of the flasks, and the fermentation was not of a foul, putrefactive nature.

TABLE 15.—*Decomposition of crimson clover cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	98.7	92.7	81.2	18.3	29.3	22.0	21.0	11.3	10.1	8.7	398.3
May 21.....	97.3	81.8	59.2	43.5	31.4	30.1	33.4	4.7	3.4	2.1	386.9
May 28.....	78.0	45.0	53.7	27.2	20.4	21.4	17.6	9.8	2.0	1.7	276.8
June 4.....	74.0	51.8	42.9	39.8	13.0	12.3	38.3	8.4	5.2	4.1	289.8
June 18.....	42.1	48.4	31.9	25.2	19.5	20.3	30.4	8.7	1.2	1.0	228.7

DAILY DECOMPOSITION (PER CENT)

May 14.....	14.61	28.33	40.36	43.06	47.40	50.66	53.77	55.44	56.94	58.22	-----
May 21.....	12.29	22.63	30.11	35.60	39.57	43.37	47.59	48.18	48.62	48.88	-----
May 28.....	11.09	17.48	25.12	28.98	31.88	34.93	37.43	38.82	39.10	39.35	-----
June 4.....	11.04	18.78	25.18	31.12	33.06	34.90	40.61	41.87	42.64	43.25	-----
June 18.....	5.91	12.70	17.18	20.72	23.45	26.30	30.57	31.79	31.96	32.10	-----

The beneficial effect of winter vetch as a green manure is clearly indicated by the results in Table 16. There was a much greater decomposition throughout the entire series of cuttings than with any of the other crops experimented with. Maximum evolution of carbon dioxide was observed in every cutting of green manure either on the first or second day of the experiment. By the tenth day the evolution of carbon dioxide had become more or less uniform over the entire series. If the decomposition is an indication of the efficiency of the green manure, the cuttings of May 14 and 21 would be the preferable ones for turning under as green manure.

Germination of native seed in the soil was more pronounced in the vetch series than with any of the other crops tried out, and nitrate production was decidedly the greatest. The soil untreated contained 3.4 mg. of nitrate in each 100 gm. of dry soil, and the nitrates produced from the treated soils ranged from 13.2 to 26 mg. It was found that the younger the grass the greater was the production of nitrate.

TABLE 16.—*Decomposition of winter vetch cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	96.8	94.8	53.7	40.6	36.4	30.2	28.6	26.4	18.7	10.9	437.1
May 21.....	105.1	56.8	49.4	55.0	48.0	36.1	28.4	26.3	20.7	18.9	444.7
May 28.....	101.6	105.7	50.8	42.7	39.9	30.0	20.6	15.2	10.0	5.1	421.6
June 4.....	54.1	81.3	34.7	23.2	18.0	16.9	15.4	13.8	12.6	11.8	281.8
June 18.....	120.7	42.9	54.7	35.6	15.7	10.1	9.3	7.4	6.3	5.9	308.6

DAILY DECOMPOSITION (PER CENT)

May 14.....	14.50	28.70	36.75	42.83	48.28	51.31	57.90	61.04	63.85	65.48	-----
May 21.....	16.58	25.54	33.33	42.00	49.57	55.21	59.75	63.90	67.16	70.14	-----
May 28.....	14.72	30.04	73.41	43.59	49.37	53.72	56.71	58.90	60.36	61.10	-----
June 4.....	7.58	18.99	23.84	27.09	29.61	31.98	34.14	36.08	37.84	39.50	-----
June 18.....	16.86	22.85	30.49	35.46	37.65	39.06	40.36	41.40	42.28	43.10	-----

Table 17 gives the total amounts of nitrate nitrogen and carbon dioxide developed over a 10-day period from the several forms of organic matter. It will be recalled that in the experiments using

Table 14 shows that the first three cuttings of oats gave the greatest rate of carbon dioxide liberation over a 10-day period. The amount of carbon dioxide evolved was greater than that from rye with a maximum liberation from the oats cut on May 28. Taking the liberation of carbon dioxide as an index to the rate of decomposition under conditions existing in the field, the third cutting of oats is the best for use as green manures.

The initial nitrogen for oats was greater than that of the rye and as a result there was greater evolution of carbon dioxide.

Soluble nitrogen determination of these samples showed the soil blank to contain 4.8 mg. of nitrate to each 100 gm. of dry soil. With the mixtures the nitrate was present in sufficient quantity to get definite results, although there was a reduction in quantity over the untreated soil. In both cases nonlegumes showed a reduction of the nitrates already present in the soil. Germination was positive, and there was no foul putrefactive odor during the decomposition as was the case when green materials fresh from the field were used.

TABLE 14.—*Decomposition of oats cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)

Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	59.9	31.8	73.1	59.1	43.0	16.4	5.2	7.9	3.3	3.2	302.9
May 21.....	115.5	39.0	40.6	32.4	31.0	24.2	11.7	8.8	4.0	2.4	309.6
May 28.....	140.6	56.7	61.6	30.8	15.6	24.6	4.7	3.0	1.8	1.4	340.8
June 4.....	50.7	44.7	64.0	30.0	28.1	26.3	7.4	10.1	6.3	3.8	271.4
June 18.....	42.9	80.8	45.7	28.4	17.6	6.8	14.5	5.4	4.6	3.6	250.3

DAILY DECOMPOSITION (PER CENT)

May 14.....	8.80	20.81	31.54	40.22	46.53	48.94	49.71	50.87	51.35	51.82	-----
May 21.....	16.23	28.74	34.45	39.00	43.36	46.76	48.40	49.64	50.20	50.54	-----
May 28.....	19.53	27.40	35.96	40.24	42.40	45.82	46.47	46.89	47.14	47.33	-----
June 4.....	7.04	13.24	22.12	26.29	30.19	33.84	34.86	34.88	37.14	37.67	-----
June 18.....	6.01	17.32	23.73	27.70	30.17	31.12	33.15	33.91	34.55	35.06	-----

The results in Table 15 show a greater evolution of carbon dioxide from clover than from either of the nonlegumes. This was especially noticeable with the cuttings of May 14 and 21. At this stage of growth the clover contained its maximum nitrogen. The greatest evolution of carbon dioxide was in the earlier days of the experiment, and it appears from these results that the young grass is decidedly the best for use as a green manure crop.

Residual nitrates were in greater quantity than in the case of the nonlegumes. The soil blank contained 0.13 mg. of nitrate nitrogen for each 100 gm. of dry soil, and there was an increase in the treated pots throughout the entire period of the experiment, the maximum nitrification being obtained with clover cut on May 21.

Germination of native seed in the soil was abundant in all of the flasks, and the fermentation was not of a foul, putrefactive nature.

TABLE 15.—*Decomposition of crimson clover cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)											
Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	98.7	92.7	81.2	18.3	29.3	22.0	21.0	11.3	10.1	8.7	398.3
May 21.....	97.3	81.8	59.2	43.5	31.4	30.1	33.4	4.7	3.4	2.1	386.9
May 28.....	78.0	45.0	53.7	27.2	20.4	21.4	17.6	9.8	2.0	1.7	276.8
June 4.....	74.0	51.8	42.9	39.8	13.0	12.3	38.3	8.4	5.2	4.1	289.8
June 18.....	42.1	48.4	31.9	25.2	19.5	20.3	30.4	8.7	1.2	1.0	228.7

DAILY DECOMPOSITION (PER CENT)											
May 14.....	14.61	28.33	40.36	43.06	47.40	50.66	53.77	55.44	56.94	58.22	-----
May 21.....	12.29	22.63	30.11	35.60	39.57	43.37	47.59	48.18	48.62	48.88	-----
May 28.....	11.09	17.48	25.12	28.98	31.88	34.93	37.43	38.82	39.10	39.35	-----
June 4.....	11.04	18.78	25.18	31.12	33.06	34.90	40.61	41.87	42.64	43.25	-----
June 18.....	5.91	12.70	17.18	20.72	23.45	26.30	30.57	31.79	31.96	32.10	-----

The beneficial effect of winter vetch as a green manure is clearly indicated by the results in Table 16. There was a much greater decomposition throughout the entire series of cuttings than with any of the other crops experimented with. Maximum evolution of carbon dioxide was observed in every cutting of green manure either on the first or second day of the experiment. By the tenth day the evolution of carbon dioxide had become more or less uniform over the entire series. If the decomposition is an indication of the efficiency of the green manure, the cuttings of May 14 and 21 would be the preferable ones for turning under as green manure.

Germination of native seed in the soil was more pronounced in the vetch series than with any of the other crops tried out, and nitrate production was decidedly the greatest. The soil untreated contained 3.4 mg. of nitrate in each 100 gm. of dry soil, and the nitrates produced from the treated soils ranged from 13.2 to 26 mg. It was found that the younger the grass the greater was the production of nitrate.

TABLE 16.—*Decomposition of winter vetch cut at different stages of growth*

DAILY PRODUCTION OF CARBON DIOXIDE (MILLIGRAMS)											
Date cut	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Total
May 14.....	96.8	94.8	53.7	40.6	36.4	30.2	28.6	26.4	18.7	10.9	437.1
May 21.....	105.1	56.8	49.4	55.0	48.0	36.1	28.4	26.3	20.7	18.9	444.7
May 28.....	101.6	105.7	50.8	42.7	39.9	30.0	20.6	15.2	10.0	5.1	421.6
June 4.....	54.1	81.3	34.7	23.2	18.0	16.9	15.4	13.8	12.6	11.8	281.8
June 18.....	120.7	42.9	54.7	35.6	15.7	10.1	9.3	7.4	6.3	5.9	308.6

DAILY DECOMPOSITION (PER CENT)											
May 14.....	14.50	28.70	36.75	42.83	48.28	51.31	57.90	61.04	63.85	65.48	-----
May 21.....	16.58	25.54	33.33	42.00	49.57	53.21	59.75	63.90	67.16	70.14	-----
May 28.....	14.72	30.04	73.41	43.59	49.37	53.72	56.71	58.90	60.36	61.10	-----
June 4.....	7.58	13.99	23.84	27.09	29.61	31.98	34.14	36.08	37.84	39.50	-----
June 18.....	16.86	22.85	30.49	35.46	37.65	39.06	40.36	41.40	42.28	43.10	-----

Table 17 gives the total amounts of nitrate nitrogen and carbon dioxide developed over a 10-day period from the several forms of organic matter. It will be recalled that in the experiments using

green manure fresh from the field not a trace of nitrogen as nitrate was found. It was also noted that the nitrate in the soil was used up. Table 17 shows that the nitrate of the soil blanks varied more or less with each of the blanks. The soil blank for the oats contained the greatest amount of nitrate and that of the clover the least.

TABLE 17.—*Parallel formation of nitrate nitrogen and carbon dioxide in soils treated with green manures*

IN MILLIGRAMS OF NITRATE NITROGEN PER 100 GRAMS OF SOIL				
Date green-manure crop was cut	Rye	Oats	Clover	Vetch
Soil blank.....	2.2	4.8	0.13	3.4
May 14.....	Trace.	1.6	13.4	26.0
May 21.....	Trace.	.5	6.0	12.0
May 28.....	Trace.	.5	1.0	18.0
June 4.....	Trace.	.5	1.0	18.6
June 18.....	Trace.	.5	1.0	13.2

MILLIGRAMS OF CARBON DIOXIDE FROM 0.5 GRAM OF ORGANIC MATTER (Ten-day totals, with soil blanks deducted)				
Soil blank.....	53.6	76.3	77.9	80.3
May 14.....	279.3	352.9	393.3	437.1
May 21.....	230.8	359.9	386.9	444.7
May 28.....	230.8	340.8	276.8	421.6
June 4.....	246.9	271.4	289.8	281.8
June 18.....	195.5	250.3	228.7	308.6

The nitrate nitrogen originally present in the soil was reduced in every instance by the nonlegumes rye and oats. An opposite effect was noted with the legumes clover and vetch. The greatest amount of nitrification was shown by vetch.

In former experiments (3) on nitrification it has been shown that there is a disappearance of nitrate nitrogen when cellulose is mixed with the soil. Such a condition will exist for probably 28 to 32 weeks, the reduction varying slightly with the soil type used. After about 32 weeks the injurious effect of the cellulose apparently passes off and nitrification then progresses slowly. It seems that there are certain types of bacteria that make use of the original nitrate of the soil for their early growth and maintenance, but after the reduction period of the first few weeks a gain is always noted.

The destruction of nitrates after cellulose is added to the soil has been reported by many investigators. Some claim that it is due to the process of denitrification, although total nitrogen analyses do not indicate a loss of nitrogen. Probably the cellulose furnishes a suitable medium for the growth of the organisms that convert nitrates into protein. In this transformation there is not any loss in total nitrogen, and in an ordinary nitrifying soil these protein compounds soon change to nitrates.

The same reduction phenomenon has been noted with rye and oats in the early stages of decomposition, but the soil recuperates much more rapidly with these forms of organic matter than with the more or less inert cellulose. It would seem, then, that legumes incorporated in the soil as green manures stimulate the formation of nitrate at a much earlier stage of their decomposition than do the nonlegumes,

which, when first turned under, apparently suppress nitrification for the first two or three weeks.

In reviewing the carbon-dioxide results, it will be seen that the young grass gave the greatest evolution of carbon dioxide when incorporated with a definite amount of soil. The readiness of decomposition was in the following order: Vetch, clover, oats, and rye. Taking the results as a whole, it appears that the green manures cut between May 14 and May 28 would be preferable as sources of organic matter for maintaining a high state of productivity.

For the nonlegumes, the ideal time for turning under would be when the plants were beginning to head. With vetch the best results are obtained when the plants are just beginning to bloom. Maximum efficiency with clover is obtained when the plants are in bloom.

SUMMARY

Pure cellulose applied to soils in different amounts restricted plant growth. This restriction was proportional to the amount of cellulose added. The addition of potassium nitrate in quantities of about 0.01 per cent failed to offset this harmful effect of the cellulose. Varying the quantity of nitrate had very little effect in counteracting the ill effects of the cellulose, beyond an 8-ton application.

Plants when grown in a well-balanced nutrient solution were healthy and vigorous, but the addition of cellulose depressed growth. Conditions were improved slightly by renewing the nutrient medium frequently. The depression in plant growth was proportional to the amount of cellulose added.

Decomposing cellulose produced hydrogen sulphide as a reduction product of the sulphates of calcium and magnesium contained in the nutrient solution.

The root systems of the plants were altered materially by a cellulose treatment, the roots becoming very much discolored and altogether unhealthy in appearance.

Crimson clover when added to sand and to Hagerstown silt loam soil in tubs and allowed to remain out of doors gave increased plant growth.

With four leading Virginia soil types, in tubs out of doors, rye in a green state depressed plant growth in the heavy clay types of soil. In the more open types of soil, a slight increase in growth was noted.

Corn grown in the field where clover was turned under gave an increased yield over corn with the clover removed for hay.

Corn was depressed in yield where rye was turned under over a period of 12 years.

Wheat grown where soy beans were cut for hay gave a much smaller yield than wheat which had soy beans turned under as a green manure.

Wheat yields were slightly increased by the turning under of buckwheat. There was a depression of yield when the buckwheat was removed for hay.

Rye, oats, clover, and vetch show a varied ratio between their nitrogen and carbon when cut on May 17. The ratio between the nitrogen and carbon is much wider for the nonlegumes than for the legumes.

The evolution of carbon dioxide from rye, oats, clover, and vetch, cut on May 17 and mixed with soil, was much greater than from the same materials which had been allowed to air-dry for the same period of time. The legumes showed a more rapid decomposition in every case than did the nonlegumes.

When green materials were allowed to decompose in the soil, no nitrates were detected after the experiment had been run for 10 days. With the same substances that had been allowed to air-dry, nitrates were present, but in much greater quantity under a legume than under a nonlegume treatment.

Drying green manures retards their decomposition when gauged by carbon dioxide liberation over a 10-day period. This retardation in decomposition may probably be due to the soluble hemicelluloses and other polysaccharides having been changed to less soluble forms.

Rye, oats, clover, and vetch, cut at different stages of development, show wide differences in their nitrogen-carbon ratio. The younger the cutting the narrower the ratio. The ratio between these two elements widens as the plant approaches maturity. Vetch gave a much narrower ratio than any of the other crops studied.

Carbon dioxide liberation from green manures cut at different stages of growth showed a rapidity of decomposition in the following order: Vetch, clover, oats, and rye. Green manure cut between May 14 and May 28 showed the highest rate of decomposition. With cellulose in a pure form the decomposition was very slow. There was a fairly good nitrification with legume treatments, but with nonlegumes the nitrates formed were in much smaller amount.

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INTERGENERIC HYBRIDS IN AEGILOPS, TRITICUM, AND SECALE¹

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INTRODUCTION

Hybridization of related but widely different forms offers an attractive field for plant improvement. With parents differing in respect to many factors, but still producing hybrids that are fully fertile, abundant material for selection is furnished. With increasing differentiation between the parents, decreasing fertility in the hybrids is likely to result until finally there is complete sterility. It is sometimes assumed that increased differentiation between the parents, up to a point just short of that at which complete sterility in the hybrids is produced, increases the opportunity for obtaining an improved form of plant. Whatever the justification for this view in other species, it has only meager support in the case of wheat. Nevertheless, the method can not be summarily dismissed as offering no possibilities, and, in fact, it has not been, for hybridization of different forms of wheat with one another and with related species proceeds increasingly. As a result, a few segregates of possible value have been obtained in interspecific, and even in intergeneric, crosses, and a considerable body of information regarding the genetic behavior and relationship of the wheat plant and its related forms is being assembled.

The results obtained in an extensive series of hybridization experiments between the several species of the genus *Triticum* and certain species of the closely related genus *Aegilops* are presented in this paper. The intention is to record the crosses made, to illustrate and describe briefly the hybrid plants obtained, and to present certain data regarding the fertility and genetics of the hybrids. In addition, previous work in the hybridization of *Aegilops* and *Triticum* is reviewed. More detailed studies of the hybrids now under way are reserved for later publication.

HISTORICAL REVIEW

Aegilops is the generic name applied to a group of plants more or less similar to wheat in appearance and taxonomic characters. The genus includes 12 or more species found growing wild in different parts of the world, but especially in the Mediterranean region. Hybrids between different species of *Aegilops* and of *Triticum* have been made by several investigators, and natural hybrids frequently have been observed. In fact, it was on account of this capacity for cross-fertilization that *Aegilops* was first brought prominently to the attention of botanists. In this a form known as *triticoïdes* was principally involved.

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In 1821 to 1824 Requien collected specimens of a plant in the environs of Avignon and Nîmes, France, which he described briefly on the herbarium sheets, applying the name *Aegilops triticoides* on account of its resemblance to cultivated wheat. Bertoloni (3, p. 788-789)² first published the description, and, considering it a new species, applied Requien's name to it. He believed that the plant reported from Italy by Gussone and Tenore under the name *A. triuncialis*, also was *A. triticoides* Req.

In 1838, Esprit Fabre found plants of *Aegilops triticoides* growing in the environs of Agde, France, and with these he immediately began experiments. This fact was communicated to the French Academy of Sciences by Auguste de Sainte-Hilaire (24) in 1839, but Fabre (6) did not publish his results until 1852.

Fabre found two kinds of plants arising from the seeds of a single spike of *Aegilops ovata*. Of this there could be no doubt, for the old spike, still well preserved, remained attached to the roots of the plants when they were pulled up and examined. One kind of plant had the short compact spike typical of *A. ovata*, and the other had a much larger spike very different from *A. ovata* but identical with the *A. triticoides* of Requien. In 1838, seed from the so-called *A. triticoides*, obtained from the typical *A. ovata*, was sown by Fabre, "in an enclosure surrounded by high walls, far from any place where cereals were cultivated, and in which there was no other gramineous plant." Successive progenies were grown for seven consecutive years in this enclosure. During this time they gradually became more like wheat, until finally he states that "these plants may be regarded as truly *Triticum*." After five years more in an open, but isolated field, the product is described as a "true wheat (*Triticum*)"; in this time *A. ovata* had never reappeared. These results led Fabre and others to believe that *A. ovata* is the progenitor of our cultivated wheat.

Fabre observed also that *Aegilops triaristata* Willd. likewise gave rise to the *triticoides* form, but he learned this too late to conduct experiments with it along with those conducted on the form arising from *A. ovata*.

Jordan (13), holding views on the immutability of species, questioned the observations and results of Fabre, claiming that he had erred in supposing that *ovata* had changed first to *triticoides* and then to *Triticum*. In his first discussion of the subject, Jordan favored the view that *triticoides* is a true species, that Fabre had been growing it throughout his experiments, and that the final supposedly wheat-like form was this species. He supposed that this had resulted from a mistake in identification at the beginning. He also advanced the alternate view, which he later (14) adopted and maintained, that *Aegilops triticoides*, the form found growing wild in France, was only a sterile modification or deformation of *A. ovata*. The fertile wheatlike plants developed by Fabre were explained as belonging to a rare, unrecorded species, probably native of the Orient, to which he gave the name *A. speltaeformis*. By supposing that confusion in identification had arisen with respect to *A. triticoides* and *speltaeformis* on the one hand, and *speltaeformis* and *Triticum vulgare* on the other, all three forms being much alike yet entirely distinct,

² Reference is made by number (italic) to "Literature cited," p. 139.

the phenomena recorded by Fabre and later by others were made to fit his own views. In 1857, Jordan (15) defended his position that *triticoïdes* is only a sterile deformation of *A. ovata*, but admitted that it is due, probably but not certainly, to hybridization. He continued to maintain that *speltaeformis* is a distinct species, and that cultivated wheat does not owe its origin to the wild *Aegilops*.

Godron (7, 8) repeatedly verified the observations of Fabre as to the origin of *Aegilops triticoïdes* from seed produced by plants of *A. ovata*. Furthermore, he observed that this form, though never abundant anywhere, was always found only on the borders of wheat fields or in their neighborhood, and only in districts where *A. ovata* occurred as a wild plant. The sudden appearance under these circumstances of a plant with the characteristics of both *Triticum* and *Aegilops*, and the fact that it seldom produced seed, led him to question the views of both Fabre and Jordan and to conclude that *triticoïdes* was a natural hybrid produced by the fertilization of *A. ovata* with wheat pollen.

In order to justify this conclusion, Godron resorted to direct experiment. In 1853, he pollinated several spikes of *Aegilops ovata* with pollen of *Triticum vulgare* and *T. spelta*, respectively, and also several spikes of *A. triaristata* with pollen of *T. durum*. As a result, one or more plants were obtained in each case which were undoubtedly hybrids. They differed in form according to the parentage, but the *A. ovata* × *T. vulgare* hybrid was practically identical with the wild *A. triticoïdes*. Apparently these plants of Godron were the first artificial hybrids ever made in the grass family, and the natural hybrids found by Requien, Fabre, and others are the first recorded instances of such hybrids in nature. Continuing his experiments, Godron (9) crossed *A. ovata* with many different wheats, mostly *T. vulgare* forms but including *T. turgidum* also. In addition he found several natural hybrids in his cultures. On account of the close resemblance of the hybrids, Godron considered the name *triticoïdes* applicable to the F_1 plants of any cross between *Aegilops* and wheat.

As a result of his experiments and observations, Godron concluded that *triticoïdes* is self-sterile, but when grown in proximity to wheat under natural conditions, or when again back crossed artificially with wheat, it produced, when fertilization occasionally occurred, the still more wheatlike form to which Jordan had given the name *Aegilops speltaeformis*. Godron grew the *A. speltaeformis* of Fabre in the twenty-sixth generation and the plants were still indefinitely fertile. The anthers were provided with pollen and emerged from the flower in anthesis, as in wheat and *A. ovata*. In 1856, Godron crossed *A. ovata* with the wheat of Agde, a bearded form, and, in 1857, backcrossed the F_1 with pollen of the same wheat. In 1858 this produced *A. speltaeformis* similar to that of Fabre, having not only the appearance but all the characters of Fabre's plant, which was being grown in the same garden. Godron grew his *speltaeformis* form for 12 years (to 1869), and it remained constant and similar to Fabre's plant. In the first year (1858) it was only slightly fertile, this agreeing with Fabre's experience, but it was very fertile in the following years. Later crosses with the wheat of Agde (10) gave a strain with persistent spikes, as Fabre also had found.

Other wheats than that of Agde when used in crosses with *Aegilops ovata* usually gave results different from the preceding, fertile constant forms seldom being produced. Apparently these latter hybrids were dependent on pollen of wheat for fertilization.

Regel (21), in 1853, appears to have been the first to question the conclusion of Fabre that he had transformed *Aegilops* into wheat. In 1856, Regel (22, 23) produced several plants of *Aegilops triticoides* from seeds of *A. ovata* which had resulted from pollination by *Triticum vulgare*. These plants were described as more like wheat than *Aegilops*, and an examination of the anthers showed only a few pollen grains, nearly all of which were shrunken and poorly developed. Apparently none was capable of fertilizing.

Groenland (11), at first working under the direction of Louis de Vilmorin, in 1855 and 1856 hybridized *Aegilops ovata* with *Triticum monococcum*, *T. sativum*, *T. spelta aristata*, and *T. turgidum*. He obtained 11 F_1 plants, all but 2 of which were sterile. The cross *A. ovata* \times *T. vulgare* was maintained to the F_3 generation, and the cross *A. ovata* \times *T. turgidum* to the F_4 generation, when sterility was complete in each case. Almost always these hybrids had returned to the paternal type, and rarely had maintained their hybrid form. From a seed produced by a natural *triticoides* plant found growing in the vicinity of Agde, Groenland developed another series of hybrids. In this series the fertility increased from generation to generation, the plants grown the fourth year (presumably the F_5) being as fertile as cultivated wheat. Plants similar to wheat, appearing in the course of the experiment, were sterile, while those retaining the hybrid or triticoidal form were more or less fertile.

Vilmorin (34), after reviewing the work of Louis de Vilmorin and Groenland (11), previously cited, reports that his own F_1 plants of the cross *Aegilops ovata* \times *Triticum vulgare* were sterile.

Bally (2) pollinated 250 flowers of *Aegilops ovata* with *Triticum vulgare*, and obtained two F_1 plants. From 80 reciprocal pollinations three F_1 plants resulted. The reciprocals were similar in appearance. All F_1 plants were sterile, their pollen being devoid of starch and otherwise not normally developed. Back crossing the F_1 plants with both parental forms failed to produce seed. In a cytological study he found that *T. vulgare* had 8 haploid chromosomes, *A. ovata* 16, and the F_1 hybrid 12 or more. The *Aegilops* chromosomes were more slender than those of *T. vulgare* and could be distinguished in the reduction division. In this connection it should be noted that both Sakamura (25) and Sax (26) report that *T. dicoccum* has 14 and *T. vulgare* 21 haploid chromosomes. Sax (27, 28) reports 14 haploid chromosomes in *A. ovata* and *A. cylindrica*. Percival (19) reports 7 haploid chromosomes in *A. cylindrica*, and 14 in *A. ovata* and *A. ventricosa*.

Tschermak (30, 31, 32) reports crossing reciprocally several species of wheat with *Aegilops ovata* and *A. cylindrica*, and also *A. ovata* with rye. In most cases the F_1 was sterile, the anthers examined being dry, failing to open, and producing no viable pollen. Nevertheless, an occasional kernel developed, especially at the tip of a head. As this occurred only on unprotected heads, natural back crossing with wheat growing near by was possible. However, a small F_2 population produced from naturally set seed of the F_1 segregated in such a way as to lead Tschermak to the conclusion that the F_1 was selfed

rather than backcrossed. Certain F_2 plants of the F_1 hybrid type also set seed when protected against cross-pollination. This evidence for F_1 self-fertility does not appear conclusive, however. Back crossing the F_1 with the parental forms very seldom gave any positive result. In contrast to the results of Groenland, the wheatlike plants among the hybrid progenies grown by Tschermak tended to be more fertile than those intermediate in form.

Cook (5) describes a wild plant found growing in Palestine which undoubtedly is a hybrid between *Aegilops ovata* and *Triticum dicoccoides* Kcke.

Blaringhem (4) crossed *Aegilops ovata* and *A. ventricosa* with *Triticum monococcum* in 1920 and 1921, but states that this was accomplished only with difficulty. Three F_1 plants, representing both crosses, were obtained, all of which were sterile, even though repeatedly back crossed with several wheats. In 1921 and 1922 Blaringhem attempted further hybridizations between *Triticum*, *Aegilops*, and *Secale*. In the *A. ventricosa* \times *S. cereale* cross two inflated ovaries were obtained. Seeds were obtained in other crosses as follows: *A. ovata* \times *T. spelta*, 1 seed and 3 inflated ovaries; *A. ovata* \times *T. vulgare*, 1 seed; *A. ovata* \times (*T. vulgare* \times *T. durum*), 4 seeds; *A. ventricosa* \times *T. spelta*, 9 seeds; and *A. ovata* \times (*T. monococcum* \times *T. durum* fixed hybrid), 13 seeds. At the time of the last report these seeds had not been grown and no data are available regarding them, including whether or not they were actually of hybrid origin. In crosses between *Aegilops* and wheat, the existence of xenia in the hybrid seed is suggested.

Percival (18) reviews much of the work which has been done on *Aegilops* hybrids. In his opinion a series of crosses initiated long ago between species of *Aegilops* and wheat of the so-called emmer species could account for the ancestry of *Triticum vulgare* and its allies.

Popova (20) reports finding hundreds of natural hybrids between *Aegilops* and *Triticum* in Turkestan, in 1921 and 1922 near Tashkent, and in 1922 also not far from Jan-Aryk. Several species of *Aegilops* are found there in abundance, growing wild on untilled land near wheat fields and to some extent in the fields themselves. Two groups of these hybrids are described, whose respective characters indicate the following parentage: *A. cylindrica* Host. \times *T. vulgare* L., and *A. crassa* Bois \times *T. vulgare* L. Complete sterility apparently existed in all but eight or nine plants, from one of which two seeds were obtained, while the others produced only one seed each. Only three F_2 plants were grown, two being like *A. cylindrica* and one like the hybrid.

METHODS AND MATERIALS

The results of an extensive series of hybridizations of two species of *Aegilops*, viz, *ovata* and *triuncialis*, with members of different groups of *Triticum*,³ and also with *Secale cereale*, are reported in this paper. *A. ovata* was crossed with one or more varieties each of *T. monococcum*, *T. dicoccum*, *T. dicoccoides*, *T. durum*, *T. polonicum*, *T. turgidum*, *T. compactum*, *T. spelta*, and *T. vulgare*, and also with *S. cereale*. *A. triuncialis* was crossed with all the forms named except *T. monococcum*. *A. ventricosa* was used in a few of the cases reported here.

³ The groups of *Triticum* named below will be referred to hereafter as species.

Aegilops is described by Ascherson and Graebner (1, p. 703-714) and others as a group of plants comprising 12 species indigenous to southern Europe, and western Asia as far as Afghanistan and Turkestan; low annuals with bent stems; spikes short, varying in shape from ovate to oblong or elongate cylindrical; spikelets with three or more flowers, glumes hard and tough, many nerved, indistinctly or not at all keeled, usually with two to many awns or teeth; lemmas leathery, with one to three awns or teeth.

Aegilops was known to the ancients, and in regions where it was indigenous it was used at a very remote period as an important article of food. At present *Aegilops* is apparently of no economic importance anywhere. One species, *A. cylindrica*, has recently been reported in the United States from Oklahoma, Kansas, and Washington. In the first two States it is found as a weed in wheat fields, apparently having been introduced with wheat seed from Russia. In Washington it is supposed to have escaped from a botanical garden.

Considerable divergence of opinion has existed among botanists with respect to the proper classification of *Aegilops*. Linnaeus (16) established *Aegilops* as a genus and named five species. Hackel (12) divided the genus *Triticum* into two sections, *Aegilops* and *Sitopyrus*, the latter including the true wheats. Ascherson and Graebner (1, p. 703-714) unite *Aegilops* and wheat in the genus *Triticum*. It appears to the present writers that *Aegilops* and *Triticum* are differentiated by characters of sufficient taxonomic importance to be considered as separate genera. This is despite the fact that hybridizations are accomplished rather readily between certain members of the two groups, and that self-fertility occasionally occurs in such hybrids.

Aegilops is regarded as a genus parallel to *Triticum*. As such, the characters of one reappear in homologous series in the other, as has been pointed out by Vavilov (33) for several genera. There is also a general resemblance between certain species in each genus. Vavilov states:

The genus *Aegilops*, which is related to *Triticum*, and grows in large quantities in natural wild conditions in southern Russia, Turkestan and Persia, as was shown by our observations, repeats in general all varieties of the genus *Triticum*. In *Aegilops squarrosa* and *Ae. cylindrica*, there are beardless as well as bearded varieties, varieties with yellow, red and black ears, hairy and smooth ears; and we know winter as well as spring varieties of these Linneons. The same division of the genus *Aegilops* into collective Linneons seems to be similar to that of *Euriticum* (cultivated wheats). The Linnean species, *Aegilops cylindrica* and *Ae. squarrosa*, are akin to *Triticum vulgare* and to other Linneons of the same group of wheat. Both are characterized in general by hollow stems, susceptibility to yellow and brown rusts (*Puccinia glumarum* and *P. triticea*), to mildew (*Erysiphe graminis*), and to smut—*Tilletia tritici*. Other Linneons, like *Aegilops triuncialis*, correspond more to *Triticum durum* or *T. monococcum* in their immunity to these parasites, and the similarity of their straw, which is full of pith, and in the absence of completely beardless varieties.

As is known, Godron artificially produced hybrids of wheat and *Aegilops*, which proves the relative affinity of these genera.

Popova (20) makes the following statement:

We find for the typical wild growing species of *Aegilops* a complete parallelism in variability with the cultivated species of the genus *Triticum*.

Stapf (29) states that he has seen specimens of *A. cylindrica* very similar in spike characters to *T. spelta*. Percival (18) furnishes illustrations of spikes of *A. cylindrica* and *T. spelta* showing the

resemblance in certain head characters. On these accounts it is difficult to taxonomically differentiate the two groups, *Aegilops* and *Triticum*. However, similar difficulty is encountered in differentiating *Triticum* from *Secale* and *Agropyron*.

The glumes of *Aegilops* are only slightly keeled or not at all, as contrasted with the always more or less distinctly keeled glumes of *Triticum*. In seed characters the different species of *Aegilops* and wheat are somewhat similar, although the seeds of all of the *Aegilops* described remain firmly inclosed in the glumes when threshed. In this they resemble einkorn, emmer, and spelt among the wheats; yet one familiar with these plants would hardly confuse the covered or naked seeds of any of the species concerned. In the general appearance and in many detailed characters of the *Aegilops* plants the same situation exists for the most part. Resemblances to *Triticum* are apparent, but usually little difficulty is experienced in properly placing the several forms.

The first plants of *Aegilops* raised in connection with the experiments reported herein belonged to the species *triuncialis*, the seeds of which were found in an introduction (Foreign Seed and Plant Introduction No. 29026) of wheat from Palestine in 1912. Since then the species *ovata*, *ventricosa*, *cylindrica*, and *speltoides* have been grown. In Figures 1 and 2 are shown representative plants of *ovata* and *triuncialis*. The leaves of these two latter species, as grown in these experiments, are narrow and abundantly hairy on both surfaces. Hairs are also found on the leaf sheaths. The height of these two species is less than half that of the average wheat. *A. triuncialis* has two to three long awns on the glumes, and very slender, lax heads with three to six spikelets each. *A. ovata* has four to seven shorter awns on the glumes, and a short, denser spike with usually three to five spikelets, only the two lower of which usually are fertile. At maturity the spikes of both of these species disarticulate at a point on the rachis below the lowest perfect spikelet. The spike remains entire and does not break up naturally into separate spikelets, as is the case in several other species of *Aegilops*.

In making crosses on *Aegilops* usually the distal spikelet of *ovata* and one or two of the uppermost spikelets of *triuncialis* were removed. Only the two lower flowers in the remaining spikelets were used for crossing, the upper flowers being entirely removed. The anthers were removed from the flowers while they were still green, and, after emasculation, the head was protected with a glassine bag. The pollen was applied to the stigmas from two to five days after emasculation. Bags were replaced after pollination was completed. In reciprocal crosses on wheat the process of hybridizing was practically the same. The number of flowers pollinated per head usually was from 18 to 24 in wheat, 4 in *ovata*, and 6 to 8 in *triuncialis*. Practically all of the crosses have been made in the greenhouses of the United States Department of Agriculture at Arlington Experiment Farm, near Washington, D. C., and nearly all of the hybrids as well as the parental forms, especially of *Aegilops*, also have been grown in these greenhouses.

The data taken on the fertility of the hybrids were confined to a consideration of the two lower flowers of each spikelet on a head.

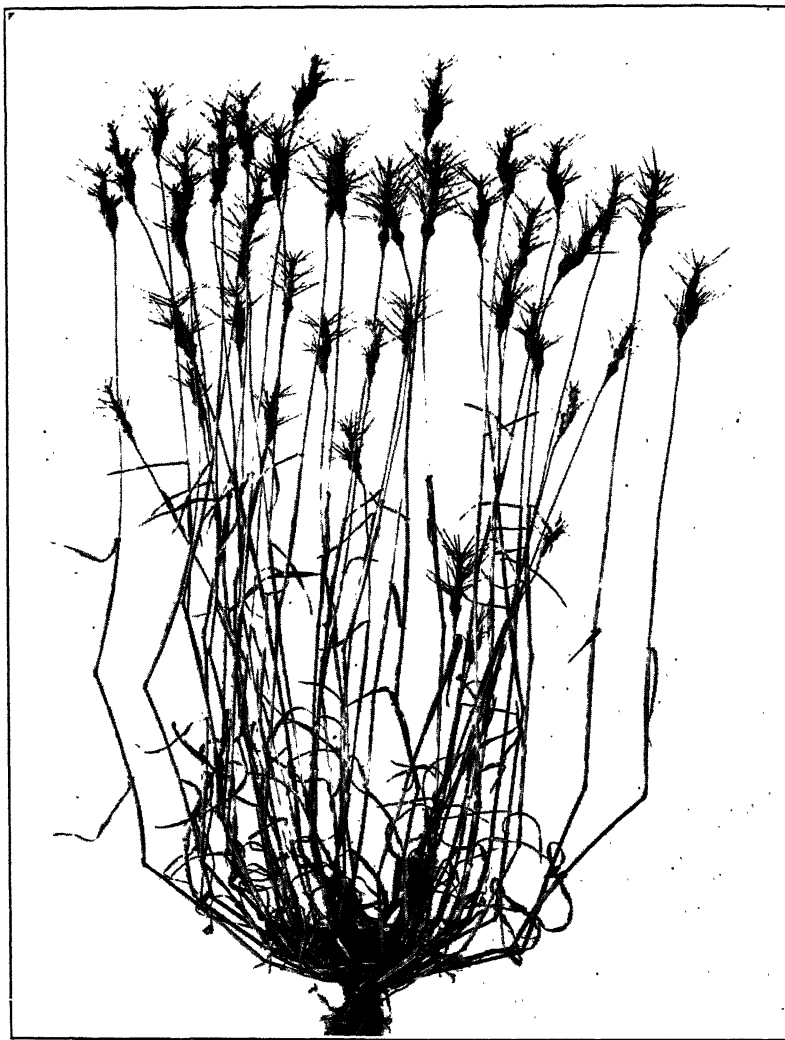


FIG. 1.—Plant of *Aegilops ovata*



FIG. 2.—Plant of *Aegilops triuncialis*

HYBRIDIZATIONS MADE

EARLIER EXPERIMENTS

The first attempt at hybridizing wheat and Aegilops in connection with these studies was made in 1913, when 146 flowers of common wheat (*Triticum vulgare*) and 8 flowers of Polish wheat (*T. polonicum*) were emasculated and pollinated with *A. triuncialis*. Six kernels were produced from the *T. vulgare* cross, but none from the *T. polonicum*. In the following year two plants were raised from these kernels, both of which were sterile. No attempts were made



FIG 3.—Spikes: A, *Aegilops triuncialis*; B, F_1 *Triticum vulgare* ♀ × *Aegilops triuncialis* ♂; C, *Triticum vulgare* (New Columbia)

to back cross the hybrids with parental forms. Figure 3 shows the first Aegilops-wheat cross made at Arlington. As far as the writers are aware this is the first report of an artificial hybrid between *A. triuncialis* and *Triticum*. Loret (17) reports that he had examined two or three incomplete specimens among the plants of M. Martin of Bédarieux which he (Loret) believed to be a hybrid between *A. triuncialis* and *T. vulgare*.

In 1918 the following crosses were attempted:

PARENTS

<i>Aegilops ovata</i> × <i>Triticum vulgare</i> :	
Flowers pollinated.....	12
Kernels obtained.....	7
<i>Aegilops triuncialis</i> × <i>Triticum vulgare</i> :	
Flowers pollinated.....	6
Kernels obtained.....	1
<i>Aegilops ovata</i> × <i>Secale cereale</i> :	
Flowers pollinated.....	6
Kernels obtained.....	4
<i>Aegilops triuncialis</i> × <i>Secale cereale</i> :	
Flowers pollinated.....	6
Kernels obtained.....	0

The four seeds obtained from *Aegilops ovata* × *Secale cereale* failed to germinate. One plant of the cross *A. triuncialis* × *Triticum vulgare*, and four plants of the *A. ovata* × *T. vulgare* cross matured in the greenhouse in 1920, all of which were again self-sterile. Backcrossing with wheat also failed.

The following crosses were made in the greenhouse in 1920:

PARENTS

<i>Aegilops ovata</i> × <i>Secale cereale</i> :	
Flowers pollinated.....	72
Kernels obtained.....	9
<i>Triticum vulgare</i> × <i>Aegilops ventricosa</i> :	
Flowers pollinated.....	26
Kernels obtained.....	4

Two F_1 plants were produced from the cross *Aegilops ovata* × *Secale cereale* and one from the cross *Triticum vulgare* × *Aegilops ventricosa*. All three hybrids were self-sterile, and backcrossing with rye and wheat failed to produce seed.

LATER HYBRIDIZATION EXPERIMENTS

In 1921, a series of experiments was initiated involving the hybridization of *Aegilops* with wheat on a more extensive scale. Crosses between most of the species of wheat and two species of *Aegilops*, viz, *ovata* and *triuncialis*, were attempted. The crosses, made principally in 1921, between *A. ovata* and *A. triuncialis*, and different species of *Triticum* are listed in Table 1, together with data on numbers of flowers pollinated, seeds obtained, and plants matured.

TABLE 1.—List of hybridizations attempted and accomplished between species of *Aegilops* and *Triticum*, with data on heads and flowers pollinated, seed obtained, and F_1 plants matured

Hybrid combination and parental wheat variety	C. I. No.	Number of heads worked	Number of flowers pollinated	Seed obtained		Number of F_1 plants matured
				Number	Per cent	
A. ovata × T. monococcum:						
Einkorn.....		9	54	7	13.0	1
A. ovata × T. dicoccum:						
Khapli.....	4013	7	34	8	23.5	5
Black Winter.....	2337	6	24	6	25.0	6
Total.....		13	58	14	24.1	11
A. ovata × T. dicoccoides:						
Wild.....	3109	8	32	7	21.9	4
A. ovata × T. durum:						
Kubanka.....	1440	5	28	7	25.0	2
Kahla.....	5529	1	6	5	83.3	3
Pentad.....	3322	2	8	5	62.5	4
Arnautka.....	1494	1	4	3	75.0	2
Total.....		9	46	20	43.5	11
T. durum × A. ovata:						
Golden Ball.....	6227	1	18	0	.0	0
Kubanka.....	1440	2	49	2	4.1	2
Total.....		3	67	2	3.0	2
A. ovata × T. polonicum:						
White Polish.....	3007	7	34	13	38.2	9
A. ovata × T. turgidum:						
Alaska.....	5988	5	26	18	69.2	9
Clackamas.....	6241	1	4	4	100.0	2
Total.....		6	30	22	73.3	11
A. ovata × T. compactum:						
Dale Glorin.....	4155	3	16	11	68.8	3
Little Club.....	4066	1	6	3	50.0	0
Mayview.....	5874	3	14	6	42.9	0
Coppel.....	3088	2	8	5	62.5	0
Big Club.....	4257	1	4	2	50.0	1
Total.....		10	48	27	56.3	4
T. compactum × A. ovata:						
Blue Chaff.....	5256	2	40	0	0	0
Jenkins.....	5177	1	16	0	0	0
Total.....		3	56	0	0	0
A. ovata × T. spelta:						
White Bearded.....	1774	5	24	8	33.3	3
Alstroum.....	1773	6	30	14	46.7	5
Total.....		11	54	22	40.7	8
A. ovata × T. vulgare:						
Grandprize.....	4876	1	6	2	33.3	1
Satisfaction.....	3588	4	20	8	40.0	2
Hard Federation.....	4980	2	10	5	50.0	1
Canadian Red.....	6282	3	14	8	57.1	4
Propo.....	1970	1	4	2	50.0	0
Sonora.....	3336	2	8	7	87.5	3
Jones Fife.....	4468	1	4	2	50.0	0
Pacific Bluestem.....	4067	1	6	2	33.3	0
Allen.....	5407	1	4	4	100.0	2
Kanred.....	5146	2	10	3	30.0	0
Champlain.....	6629	2	8	5	62.5	1
.....	4782	1	6	4	66.7	0
F_1 Fultz × C. I. No. 6193.....		1	4	4	100.0	1
Preston.....	3328	1	4	4	100.0	0
Purplestraw.....	1915	3	12	11	91.7	3
Stanley.....	4796	1	4	4	100.0	3
Kofod.....	4337	1	4	4	100.0	3
Ledoga.....	4795	2	8	7	87.5	5
Humpback.....	3690	1	4	4	100.0	2
Trumbull.....	5657	1	4	3	75.0	1

TABLE 1.—*List of hybridizations attempted and accomplished between species of Aegilops and Triticum, with data on heads and flowers pollinated, seed obtained, and F₁ plants matured—Continued*

Hybrid combination and parental wheat variety	C. I. No.	Number of heads worked	Number of flowers pollinated	Seed obtained		Number of F ₁ plants matured
				Number	Per cent	
<i>A. ovata</i> × <i>T. vulgare</i> —Continued.						
Rising Sun.....	5977	2	8	0	0	0
Red Chaff.....	4241	1	4	1	25.0	0
2090a-1.....		1	4	4	100.0	1
Total.....		36	160	98	61.3	33
<i>T. vulgare</i> × <i>A. ovata</i> :						
Marquis.....	3641	1	24	3	12.5	3
Walker.....	6445	1	14	0	0	0
Total.....		2	38	3	7.9	3
<i>A. triuncialis</i> × <i>T. dicoccum</i> :						
Khapli.....	4013	3	20	5	25.0	5
<i>A. triuncialis</i> × <i>T. dicoccoides</i> :						
Wild.....	3109	3	22	9	40.9	7
<i>T. dicoccoides</i> × <i>A. triuncialis</i> :						
Wild.....	3109	2	30	0	0	0
<i>A. triuncialis</i> × <i>T. durum</i> :						
Peliss.....	1584	1	10	8	80.0	4
<i>T. durum</i> × <i>A. triuncialis</i> :						
Kubanka.....	1440	1	26	6	23.1	0
<i>A. triuncialis</i> × <i>T. polonicum</i> :						
White Polish.....	3007	1	6	1	16.7	1
<i>A. triuncialis</i> × <i>T. turgidum</i> :						
Alaska.....	5988	1	6	2	33.3	2
Titantic.....	5535	1	8	0	0	0
Total.....		2	14	2	14.3	2
<i>A. triuncialis</i> × <i>T. compactum</i> :						
Dale Gloria.....	4155	1	6	5	83.3	5
<i>T. compactum</i> × <i>A. triuncialis</i> :						
Blue Chaff.....	5256	1	4	1	25.0	0
Jenkin.....	5177	1	12	2	16.7	0
Dale Gloria.....	4155	1	10	1	10.0	0
Total.....		3	26	4	15.4	0
<i>A. triuncialis</i> × <i>T. spelta</i> :						
White Bearded Spelt.....	1774	2	12	4	33.3	4
<i>A. triuncialis</i> × <i>T. vulgare</i> :						
Prelude.....	4323	1	4	4	100.0	1
Hard Federation.....	4980	1	8	6	75.0	5
Canadian Red.....	6282	1	8	5	62.5	5
Sonora.....	3036	2	12	6	50.0	5
Grandprize.....	4876	1	6	4	66.7	2
Rink.....	5868	1	6	2	33.3	1
Total.....		7	44	27	61.4	19
<i>T. vulgare</i> × <i>A. triuncialis</i> :						
Rising Sun.....	5977	1	22	3	13.6	1
Walker.....	6445	1	22	0	0	0
Purplestraw.....	1915	1	18	2	11.1	1
Total.....		3	62	5	8.1	2

HYBRIDS BETWEEN *AEGILOPS OVATA* AND *TRITICUM* SPECIES

In Table 1 the crosses made between *Aegilops ovata* and different wheat forms are listed first, and following these are the crosses in which *A. triuncialis* was used. The different combinations will be considered in the order in which they appear in the table, the first being that between *A. ovata* and *Triticum monococcum*. Only one variety of *monococcum* was used, pollen of which was applied to 54 flowers of *ovata*. As a result seven seeds were obtained, from which

one plant was produced. A spike from one of these plants, together with spikes of the parents, are shown in Figure 4. The resemblance of this F_1 plant to the *monococcum* parent is quite striking. The F_1 plant was 26 inches in height, and the *monococcum* parent 30 inches.

Two varieties of emmer (*Triticum dicoccum*), Khapli and Black Winter, were used as pollen parents in crosses with *A. ovata*. A total of 58 flowers was pollinated, of which 14, or 24.1 per cent, produced seed. From these, 11 F_1 plants were produced, a spike from one of which is shown in Figure 5, together with a spike of Khapli emmer. This is one of the few hybrids between *A. ovata* and Triticum from which seeds were obtained by selfing.



FIG. 4.—Spikes: A, *Aegilops ovata*; B, F_1 *Aegilops ovata* \times *Triticum monococcum* *vulgare* δ ; C, *Triticum monococcum* *vulgare*

When *Aegilops ovata* was crossed with the so-called "wild wheat of Palestine" (*Triticum dicoccoides*), seven, or 21.9 per cent, of the 32 flowers pollinated produced seeds. From these, four F_1 plants were grown. A spike of one of these is shown in Figure 5 together with a spike of *T. dicoccoides*.

Four varieties of durum wheat (*Triticum durum*) were used as the pollen parent in crosses with *Aegilops ovata*, 46 flowers being pollinated of which 20, or 43.5 per cent, produced seeds. Eleven F_1 plants were obtained, a spike of one of which is shown in Figure 6, together with spikes of the parental forms, *ovata* and Kubanka durum. The F_1 of this cross was vigorous, and was self-fertile to a slight degree. The F_1 of the cross in which Arnautka durum was used as the pollen parent was considerably more self-fertile than the cross with Kubanka.

In the reciprocal of the above cross, 67 flowers of 2 varieties of durum pollinated by *Aegilops ovata* produced only 2 seeds, or a percentage of 3.0. These were produced by Kubanka, the crosses on Golden Ball being unsuccessful.

Polish wheat (*Triticum polonicum*) was used to pollinate 34 flowers of *A. ovata*, of which 13, or 38.2 per cent, produced seeds. From these



FIG. 5.—Spikes: A, *Triticum dicoccum* (Khapli); B, F_1 of *Aegilops ovata* ♀ × *Triticum dicoccum* ♂; C, F_1 of *Triticum dicoccoides* ♀ × *Aegilops ovata* ♂; D, *Triticum dicoccoides*

nine F_1 plants were obtained. A spike of one of these is shown in Figure 7, together with a spike of each of the parents. The influence of the Polish parent in increasing the glume length of the hybrid is apparent when the F_1 of this cross is compared with the F_1 of other combinations illustrated. The F_1 was almost completely sterile. White Polish, the wheat parent, however, was partially sterile when grown in the same environment as the hybrids.

Two varieties of *Triticum turgidum*, Alaska and Clackamas, were used in crosses with *Aegilops ovata*, a total of 30 flowers being pollinated. Of these 22, or 73.3 per cent, produced seed. Eleven F_1



FIG. 6.—Spikes: A, *Aegilops ovata*; B, F_1 of *Aegilops ovata* ♀ × *Triticum durum* ♂; C, *Triticum durum* (Kubanka)

plants were obtained, a spike of one of which is shown in Figure 8, together with spikes of the parental forms, *A. ovata* and Alaska wheat. The F_1 was self-sterile, but set seed when pollinated with wheat.

Five varieties of club wheat (*Triticum compactum*) were used as the pollen parent in crosses with *Aegilops ovata*. From the 48 flowers pollinated, 27 seeds were obtained, or 56.3 per cent. A spike of the



FIG. 7.—Spikes: A, *Aegilops ovata*; B, F_1 of *Aegilops ovata* \times *Triticum polonicum* δ ; C, *Triticum polonicum* (White Polish)

F_1 and of each of the parents, *ovata* and Dale Gloria, is shown in Figure 8. The lax hybrid head shows little or no influence of the compact-headed wheat parent, in respect to head shape. The F_1 plant was dwarfed and stunted in growth and late in maturing. The fully developed plant was shorter even than the *ovata* parent,

and it was completely sterile. Two different varieties of club wheat were used as the female parent in crosses with *ovata*, but no seed was obtained in 56 attempts.

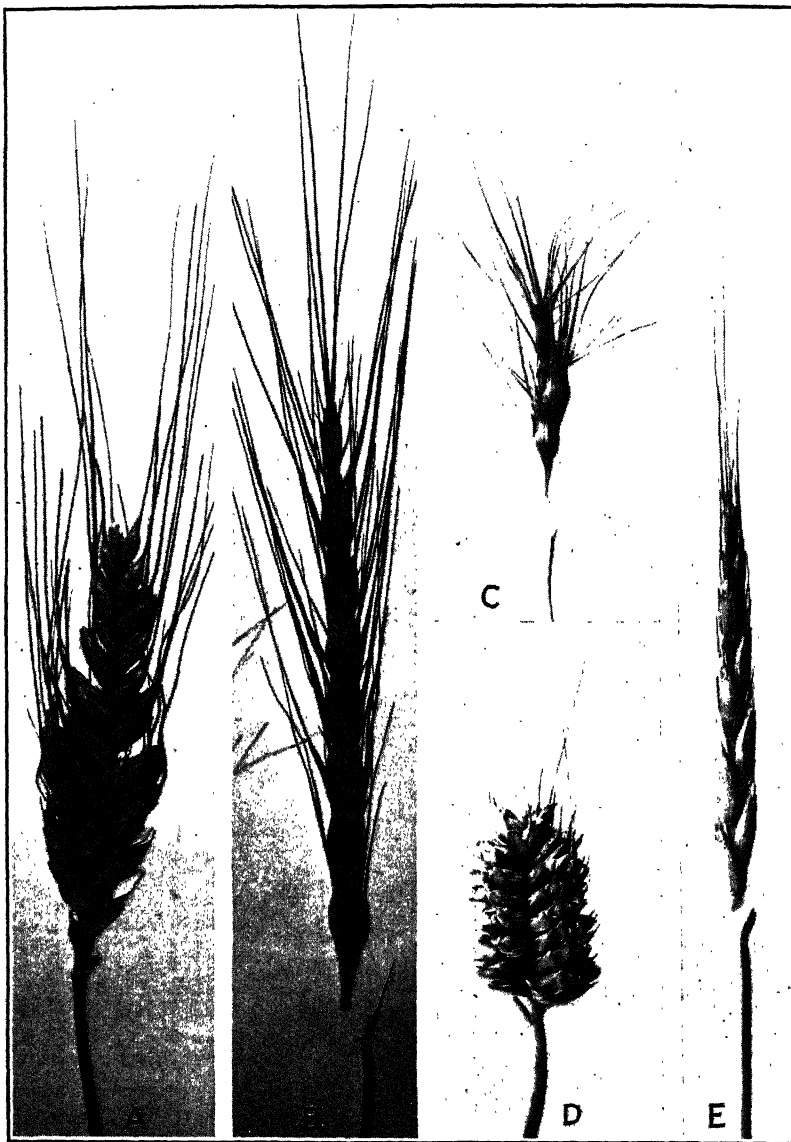


FIG. 8.—Spikes: A, *Triticum turgidum* (Alaska); B, F_1 of *Aegilops ovata* ♀ × *Triticum turgidum* ♂; C, *Aegilops ovata*; D, *Triticum compactum* (Dale Gloria); E, F_1 *Aegilops ovata* ♀ × *Triticum compactum* ♂.

Two varieties of spelt (*Triticum spelta*), White Bearded and Alstrom, were used in crosses on *Aegilops ovata*. From the 54 flowers pollinated, 22 seeds were obtained, a percentage of 40.7. Eight F_1 plants were produced, of which a spike is shown in Figure 9, together with

Aegilops ovata and White Bearded spelt, the parents. The F_1 of this hybrid was self-sterile, but a few seeds were produced when wheat (*Triticum vulgare*) pollen was used for fertilization.

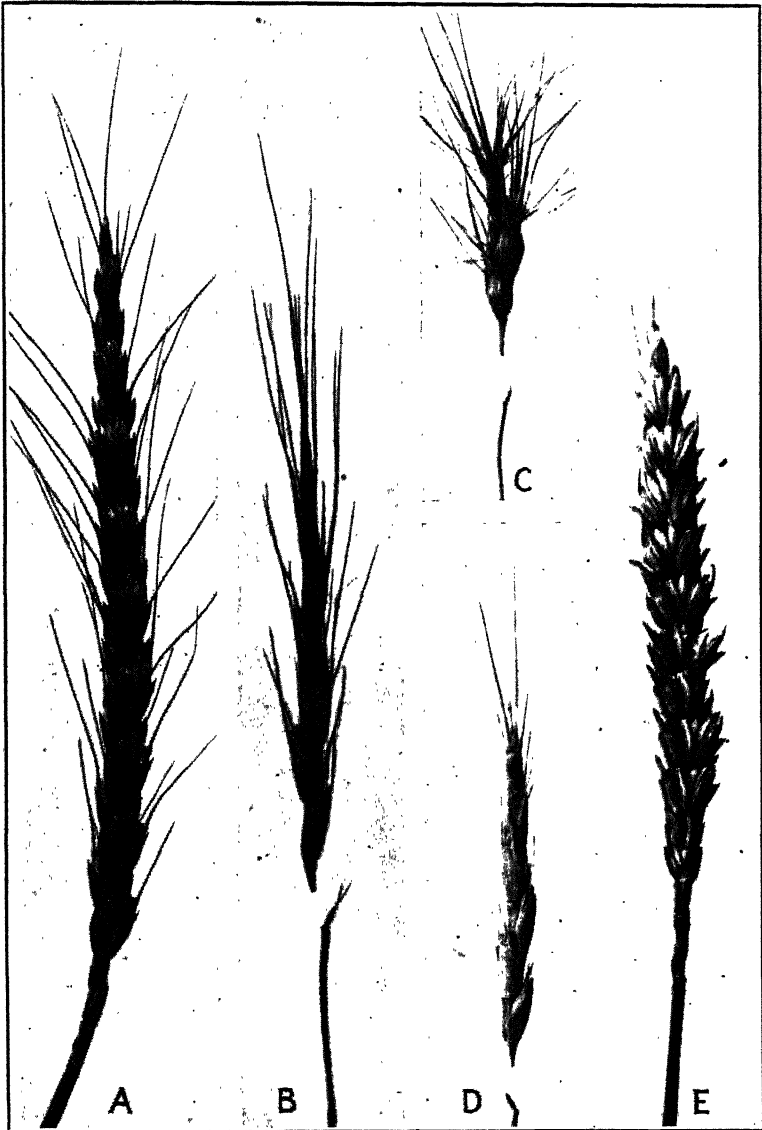


FIG. 9.—Spikes: A, *Triticum spelta* (White Bearded); B, F_1 of *Aegilops ovata* ♀ × *Triticum spelta* ♂; C, *Aegilops ovata*; D, F_1 of *Aegilops ovata* ♀ × *Triticum vulgare* ♂; E, *Triticum vulgare* (Purple-straw)

The most extensive series of hybrids between *Aegilops ovata* and the different wheat groups is that in which *Triticum vulgare* was used. Of the latter, 23 different strains or varieties were used as the pollen parent in crosses on *ovata*. A wide range of types is represented

in these strains, including winter and spring, white-kerneled and red-kerneled, hard red and soft red, varieties adapted to the most diverse climates and conditions, and varieties differing in many morphological characters. Of the 160 *ovata* flowers pollinated with these various strains, 98, or 61.3 per cent, produced seed. In the case of only one strain was no seed produced, all other crosses resulting in from 25 to 100 per cent fertility. The reciprocal cross, in which *ovata* furnished the pollen, was attempted on only 38 flowers of two varieties of *T. vulgare*. From these only 3 seeds were obtained, or 7.9 per cent.

From the 101 seeds produced in the crosses between *Aegilops ovata* and *Triticum vulgare*, 36 F_1 plants were grown. A spike of the *ovata* \times Purplestraw cross, and of each of the parents, is shown in Figure 9.

HYBRIDS BETWEEN AEGILOPS TRIUNCIALIS AND TRITICUM SPECIES

Aegilops triuncialis was crossed with one variety of *Triticum dicoccum*, the Khapli emmer. From the 20 flowers pollinated, five seeds were obtained, a percentage of 25. From these seeds 5 plants were obtained. A spike of one of these F_1 plants and spikes from the two parents are shown in Figure 10. This hybrid showed slight self-fertility.

Reciprocal crosses were attempted between *Aegilops triuncialis* and the "wild wheat of Palestine" (*Triticum dicoccoides*). From the 22 flowers pollinated by the wild emmer, 9 seeds were obtained, or 40.9 per cent. No seed was obtained from the reciprocal cross. From the nine seeds, seven F_1 plants were produced, a spike of the F_1 being shown in Figure 10 together with spikes of the parental forms. This hybrid was exceptionally vigorous and produced selfed seed.

Durum wheat (*Triticum durum*) was used in reciprocal crosses with *Aegilops triuncialis*. When Peliss durum was used as the pollen parent, 8 seeds were obtained from 10 flowers pollinated, a percentage of 80. From these, four plants were produced. In the reciprocal cross, in which Kubanka durum was used as the female parent, 26 flowers produced 6 seeds, a percentage of 23.1. From these seeds no plants were obtained. A spike of the F_1 hybrid between *triuncialis* and Peliss durum, and spikes of the parents, are shown in Figure 11. The F_1 of this cross also was vigorous and self-fertile. The F_1 spike was somewhat speltlike in appearance.

Only one seed was obtained from six flowers of *Aegilops triuncialis* pollinated by Polish wheat (*Triticum polonicum*), a percentage of 16.7. From this single seed a plant was grown, which was completely sterile. The spike of this F_1 plant, shown with parental spikes in Figure 11, exhibits some influence of the Polish parent in the character of the glumes.

Two varieties of *Triticum turgidum*, Alaska and Titanic, were used for pollinating *Aegilops triuncialis*. None of the 8 flowers pollinated by Titanic produced seed, but 2 seeds were obtained from 6 flowers pollinated by Alaska, a percentage of 33.3. From these seeds 2 plants were grown, which were highly self-fertile, producing a total of 44 seeds. Spikes of the hybrid and of the parents are shown in Figure 12.

Three varieties of club wheat (*Triticum compactum*) were used in crosses with *Aegilops triuncialis*. When Dale Gloria was used as the pollen parent, five seeds were produced by six flowers pollinated, a percentage of 83.3. This variety and two others when used as

the female parents in crosses with *triuncialis*, produced four seeds from 26 flowers, a percentage of 15.4. From these four seeds no plant was obtained, but five plants were obtained from the five seeds obtained in the *triuncialis* × Dale Gloria cross. A spike from one of these, together with the parents, is shown in Figure 12. The



FIG. 10.—Spikes: A, *Aegilops triuncialis*; B, F₁ of *Aegilops triuncialis* ♀ × *Triticum dicoccum* ♂; C, *Triticum dicoccum* (Khapli); D, F₁ of *Aegilops triuncialis* ♀ × *Triticum dicoccoides* ♂; E, *Triticum dicoccoides*.

influence of the wheat parent on head shape is evident in this F₁, the spike obtained in this cross being the most compact obtained in any of the crosses with *triuncialis*, as the wheat parent is also the most compact wheat used. The F₁ of this cross was quite vigorous and was both self-fertile, although only slightly so, and productive of seed when backcrossed with wheat pollen.

White Bearded spelt was the only variety of *Triticum spelta* used in a cross with *Aegilops triuncialis*, 12 flowers of the latter producing 4 seeds, a percentage of 33.3. From these seeds four plants were produced, of which a spike, together with the parents, is shown in Figure 13. This hybrid showed slight fertility, both when selfed and when backcrossed with pollen of common wheat.



FIG. 11.—Spikes: A, *Aegilops triuncialis*; B, F₁ of *Aegilops triuncialis* ♀ × *Triticum durum* ♂; C, *Triticum durum* (Peliss); D, F₁ of *Aegilops triuncialis* ♀ × *Triticum polonicum* ♂; E, *Triticum polonicum* (White Polish).

Aegilops triuncialis was pollinated with six varieties of common wheat (*Triticum vulgare*). These included both spring and winter, and red-grained and white-grained forms. Of 44 flowers pollinated by these six varieties, 27, or 61.4 per cent, produced seeds, from which 19 plants were grown. One or more seeds were produced in the case of each variety used.



FIG. 12.—Spikes: A, F_1 of *Aegilops triuncialis* ♀ × *Triticum compactum* ♂; B, *Triticum compactum* (Dale Gloria); C, *Aegilops triuncialis*; D, F_1 of *Aegilops triuncialis* ♀ × *Triticum turgidum* ♂; E, *Triticum turgidum* (Alaska).

Pollen from *Aegilops triuncialis* was used on 62 flowers of three varieties of common wheat, 5 seeds being produced, a percentage of 8.1. The Walker variety did not produce seed. Only two plants



FIG. 13.—Spikes: A, *Aegilops triuncialis*; B, F₁ of *Aegilops triuncialis* ♀ × *Triticum spelta* ♂; C, *Triticum spelta* (White Bearded); D, F₁ of *Aegilops triuncialis* ♀ × *Triticum vulgare* ♂; E, *Triticum vulgare* (Purplestraw).

were produced from these seeds. An F₁ spike of the cross between *A. triuncialis* and the soft red winter variety Purplestraw, and spikes of the two parents, are shown in Figure 13. This cross produced vigorous F₁ plants but they were self-sterile.

SUMMARY OF AEGILOPS-TRITICUM CROSSES MADE

Summarizing Table 1, it is found that 516 flowers on 109 spikes of *Aegilops ovata* pollinated by nine species of *Triticum* produced 230 seeds, or 44.6 per cent of successful pollinations. In the crosses with *A. ovata* the highest percentage, 73.3, was obtained when *T. turgidum* pollen was used: the second highest, 61.3, with *T. vulgare*; and the third highest, 56.3, with *T. compactum*. *T. durum*, *T. polonicum*, and *T. spelta* pollinations were each successful in about 40 per cent of the cases, while the percentages of successful pollinations with *T. dicoccum* and *T. dicoccoides* were 24.1 and 21.9, respectively. The lowest percentage of successful pollinations was with *T. monococcum*, in which cross only 13 per cent of the *A. ovata* flowers produced seed. On account of the limited numbers of flowers pollinated in most cases, and the more or less varying conditions under which such work usually must be done, there is perhaps little significance in the variation in percentages of successful pollinations when the different species were used.

When *Aegilops triuncialis* was used as the female parent, 134 flowers on 20 heads, pollinated by the same species of *Triticum* as used in the above crosses, produced 61 seeds, or a percentage of 45.5 of the total pollinations. In most cases few pollinations of *triuncialis* were made. The results obtained from *triuncialis* with the different species of wheat, therefore, are even less comparable than in the crosses with *A. ovata*. The percentages of the pollinations which were successful varied from about 80 per cent for both *T. compactum* and *T. durum* to 14.3 per cent for *T. turgidum*. In this latter case only 14 flowers were pollinated, and so it can not be concluded that this combination is essentially different from the others.

Reciprocal crosses in which wheat was used as the female parent were much less successful. From 161 flowers on 8 heads of the 3 *Triticum* species, *durum*, *compactum*, and *vulgare* pollinated by *Aegilops ovata*, only 5 seeds were obtained, or a percentage of 3.1. From 144 flowers on 9 heads of 4 *Triticum* species, *dicoccoides*, *durum*, *compactum*, and *vulgare*, pollinated by *A. triuncialis*, 15 seeds were obtained, or a percentage of 10.4. The poor results in these reciprocal crosses, in which wheat is used as the female parent, in contrast to its use as the pollen parent, are attributed partly to the fact that all the flowers of a single wheat head are not receptive at the time of the single pollination. It is likely, however, that some incompatibility between *Aegilops* and wheat exists in the case when wheat is used as the female parent that does not exist when *Aegilops* is so used, just as appears to be the case in wheat and rye when rye is used as the female parent.

It is evident from the data in Table 1 that, for the most part, the different species and varieties of wheat used cross readily with *Aegilops ovata* and *A. triuncialis* when the latter are used as the female parents. The largest number of crosses with any species of wheat were made with *vulgare*, in both cases. The percentages of success attained when *A. ovata* and *A. triuncialis* were pollinated with *T. vulgare* pollen were 61.25 and 61.36 per cent, respectively. In crosses made in the greenhouse the same year between different varieties of *vulgare* wheat, only 35 per cent of the pollinations made resulted in the production of seed. However, it should not be concluded that *A. ovata* and *A. triuncialis* are more compatible with

T. vulgare pollen than is the pollen of one variety of *T. vulgare* with another. Under favorable greenhouse conditions, *A. ovata*, usually having only three spikelets to the head, completes its blooming in two days and occasionally in one, while *A. triuncialis*, which has five or six spikelets, requires a slightly longer period. In wheat grown under similar conditions, however, the complete blooming of all flowers on a single head commonly requires a period of five or more days, although the removal of flowers and spikelets in preparation for pollination somewhat reduces this period for any head. All of the flowers that were used for crossing on a head of *A. ovata*, therefore, are receptive at about the same time, and all flowers pollinated on a head produced seed in several cases (Table 1). The larger number of spikelets of *A. triuncialis* utilized in crossing, however, may result in some of the flowers on a head not being receptive at the time of a single application of pollen. It is even more probable that some of the 18 to 24 flowers utilized for crossing in a head of *T. vulgare* would not be receptive at the time of a single pollination, especially under greenhouse conditions.

HYBRIDS BETWEEN SECALE CEREALE AND AEGILOPS SPECIES

Crosses were made between rye (*Secale cereale*) and three species of Aegilops, viz, *ovata*, *triuncialis*, and *ventricosa*, rye being used as the pollen parent in all cases. The results of these crosses are given in Table 2. From 180 flowers of *ovata* crossed with rye, 51 malformed seeds were produced, a percentage of 28.3. Very few of these seeds germinated, and only two plants were matured. Spikes of the F_1 and of the parents are shown in Figure 14, and enlarged spikelets in Figure 15. The F_1 was completely sterile. The pubescent peduncle of rye was dominant in the F_1 ; spikelet characters are somewhat intermediate.

TABLE 2.—List of hybridizations attempted and accomplished between *Secale cereale* and three species of Aegilops—*ovata*, *triuncialis*, and *ventricosa*

Hybrid combination and rye variety	C. I. No.	Number of heads worked	Number of flowers pollinated	Seed obtained		Number of F_1 plants matured
				Number	Per cent	
Aegilops ovata × Secale cereale:						
Unknown.....			180	51	28.3	2
A. triuncialis × S. cereale:						
Von Rümker.....	133	1	6	0	0	0
Rosen.....	195	3	18	12	66.7	1
Total.....		4	24	12	50.0	1
A. ventricosa × S. cereale:						
Unknown.....			54	28	51.9	0

Two varieties of rye were used in crosses with *Aegilops triuncialis*. The 24 flowers pollinated produced 12 seeds, a percentage of 50. From these seeds only one plant was obtained, of which a spike, together with those of parents, is shown in Figure 14. This plant was also completely sterile, and the pubescent peduncle of rye again was dominant. The spike of the *A. triuncialis* × rye hybrid is longer and more lax than that of *A. ovata* × rye, but in other respects they are similar.

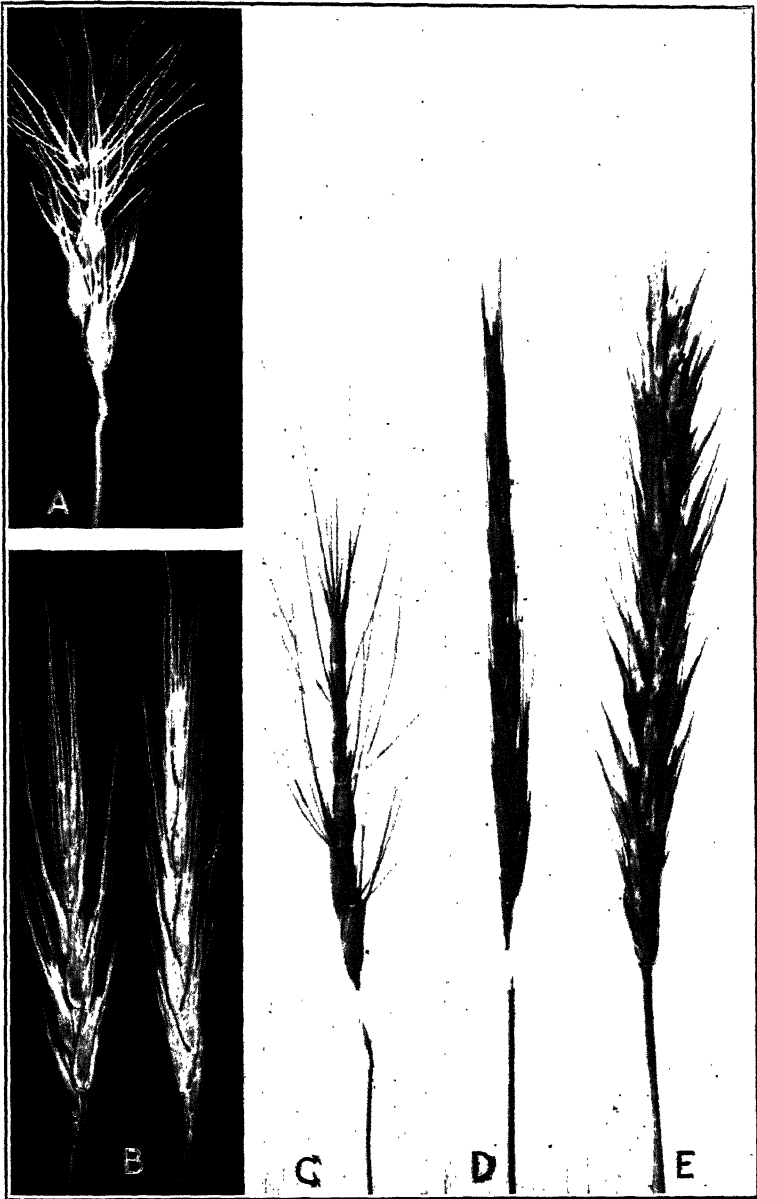


FIG. 14.—Spikes: A, *Aegilops ovata*; B, F₁ of *Aegilops ovata* ♀ × *Secale cereale* ♂; C, *Aegilops triuncialis*; D, F₁ of *Aegilops triuncialis* ♀ × *Secale cereale* ♂; E, *Secale cereale*

From 54 pollinations of *Aegilops ventricosa* with rye 28 seeds were obtained, none of which germinated.

Although 91 seeds were produced in these crosses between rye and *Aegilops*, and all were planted, only 3 plants have been grown. In all crosses involving *Aegilops* and rye, the seeds obtained were con-

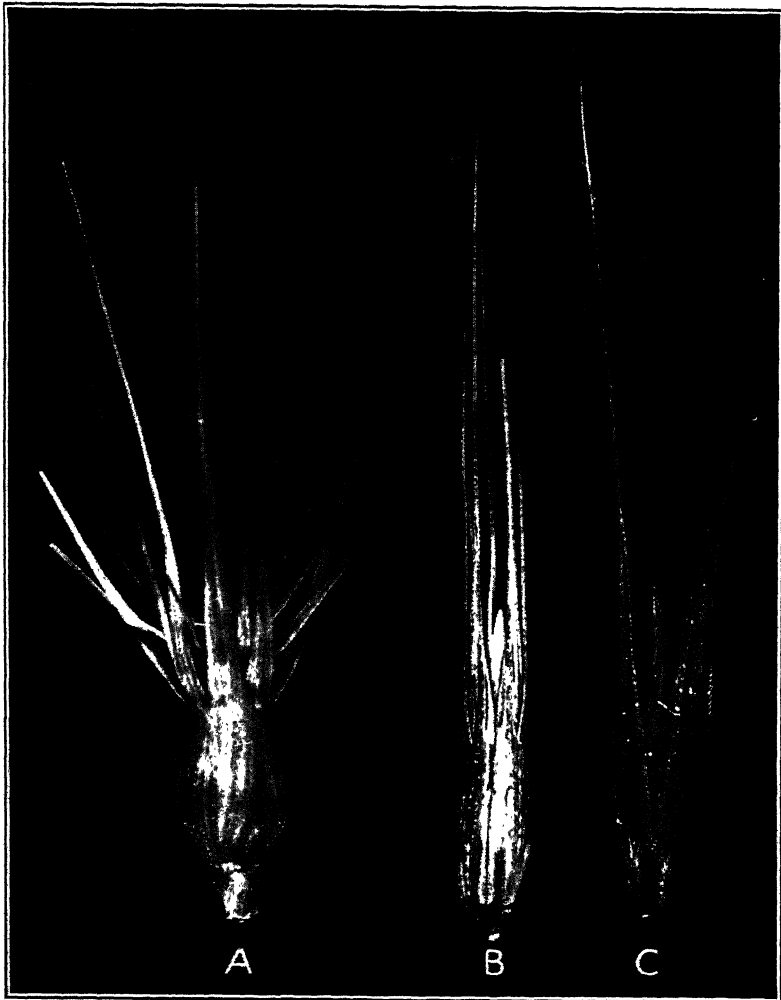


FIG. 15.—Spikelets: A, *Aegilops ovata*; B, F₁ of *Aegilops ovata* ♀ × *Secale cereale* ♂; C, *Secale cereale*

siderably shrunken and lacking in endosperm development. These hybrid seeds germinated very poorly, and the few plants obtained were completely sterile. Hybrids between *Secale* and *Aegilops* have never been carried beyond the F₁ generation, so far as known to the present writers.

AEGILOPS OVATA POLLINATED WITH BARLEY

It is also of interest to note that from 141 pollinations of *Aegilops ovata* with cultivated barley, 26 kernels were obtained, all of which failed to germinate. These kernels likewise showed very poor endosperm development, and no embryos were observed. It appears that in these crosses of *Aegilops* with barley, and to some extent with rye, a limited endosperm growth has been stimulated by the pollen applied, but that this stimulation did not result in embryo formation. At least embryo development did not proceed very far, and endosperm development also soon ceased.

THE F₁ GENERATION OF AEGILOPS-TRITICUM HYBRIDS

Two hundred and thirty-five seeds obtained from reciprocal crosses between *Aegilops ovata* and different species of *Triticum*, and 76 seeds obtained from reciprocal crosses between *A. triuncialis* and different species of *Triticum*, were sown in 6-inch pots in the greenhouse in December, 1922. Germination of the seed was poor, and there was a small additional loss after germination, the plants dying before the third leaf was produced. However, 97 plants matured from crosses between *A. ovata* and *Triticum*, and 49 from crosses between *A. triuncialis* and *Triticum*, this being 41.3 per cent and 64.5 per cent, respectively, of the seeds planted. All of the plants which matured proved to be true hybrids.

The F₁ plants matured in each of the following crosses consistently showed dwarfness and a reduced number of culms as compared with the normal F₁ plant of other crosses:

Aegilops ovata × Purplestraw (*Triticum vulgare*).

Aegilops ovata × Big Club and Dale Gloria (*Triticum compactum*).

Aegilops ovata × White Bearded spelt (*Triticum spelta*).

When the same wheats (with the exception of Big Club, which was not used) were crossed with *A. triuncialis*, the hybrids showed normal vigor. As great variability in vigor of growth is found in crosses between *Aegilops* and the several *Triticum* species used.

GENETICS OF THE F₁ GENERATION

Requien (3, p. 788-789) gave the name *Aegilops triticoides* to the plant occasionally found in the southern part of France, which later was shown to be the natural hybrid between *Aegilops* and wheat. Godron (9) applied this name to all F₁ hybrids between wheat and *Aegilops*. He did this because all of the F₁ *Aegilops*-wheat plants which he had observed were similar in appearance. Certain characters such as the shape of the head, form of the spikelets, spininess of the outer glumes, and sterility, were common to all his F₁ hybrids. He observed, however, that an awnless wheat used as a parent produced a hybrid with half-abortive awns, while an awned wheat resulted in a long-awned hybrid. Certain other differences observed were also ascribed to similar differences in the wheat parent. The hybrids of *A. ovata* × *Triticum* grown in connection with the present investigations closely resembled one another, differing only in such characters as were possessed by the wheat parent. For example, an awned wheat crossed with *A. ovata* gave a fully-awned F₁, while an awnless wheat when crossed with *A. ovata* produced a hybrid with the awns greatly reduced. The awns of the *A. ovata* × *T. durum* hybrid were longer than those of the *A. ovata* × *T. vulgare* hybrid, etc. The F₁ plants of *A. triuncialis* × wheat were distinguishable from

those of *A. ovata* \times wheat principally by having a more slender head, more spikelets, and a smaller number of awns or teeth on the glumes. Otherwise the two groups of hybrids were similar and at times indistinguishable. The nature of the F_1 inheritance of 10 characters differentiating the *Aegilops* and *Triticum* parents is shown in Table 3.

TABLE 3.—Nature of inheritance in the F_1 plant of characters differentiating the *Aegilops* and *Triticum* parents

Characters	<i>Aegilops</i>	F_1	<i>Triticum</i>
1. Sheath and leaves.....	Hairy.....	Intermediate.....	Few to none.
2. Tillers.....	Many.....	Many.....	Few.
3. Height of plant.....	Short.....	Intermediate.....	Tall.
4. Culms.....	Bent.....	Semierect.....	Erect.
5. Spikelets per head.....	Few.....	Intermediate.....	Many.
6. Awns or beaks on the glume.....	2-7.....	2-4.....	1.
7. Glume.....	Spiny.....	Spiny.....	Smooth.
8. Awns or teeth on lemma.....	2-3.....	1-3.....	1.
9. Glumes.....	Rounded.....	Keeled.....	Keeled.
10. Disarticulation of head.....	Deciduous.....	Deciduous.....	Persistent.

In Figures 3 to 13, inclusive, showing the heads of the parents and the F_1 plants, a general resemblance of the latter to the wheat parent may be observed. It is evident, however, from Table 3 and also from the various illustrations, that the *Aegilops* characters predominate in the F_1 .

In crosses between wheat varieties, dominance usually occurs in the following spike characters: Awnlessness to beardedness, red glumes to white glumes, pubescent glumes to glabrous glumes, and club to fusiform shape of spike. With the exception of the last-named character, dominance was expressed in the *Aegilops* \times *Triticum* crosses in the same manner as in crosses between wheat varieties. In the cross between varieties of wheat with clavate and fusiform spikes, the F_1 usually has a clavate or partially clavate spike. The *A. ovata* \times *T. compactum* (Big Club and Dale Gloria) cross produced F_1 plants none of which showed any indication of clavateness (fig. 8). When *A. triuncialis* was crossed with Dale Gloria, however, the influence of the *T. compactum* parent was evident in the F_1 , the spike being somewhat clavate in shape (fig. 12).

When *Aegilops ovata* and *A. triuncialis* were grown in the greenhouse, the number of culms produced was very variable, ranging in *ovata* from 10 to 90, with an average of 40; and in *triuncialis* from 10 to 70, with an average of 30. Common wheat (*Triticum vulgare*) grown under similar conditions in the greenhouse produced from 2 to 10 culms, with an average of 4. F_1 plants of the cross *ovata* \times *vulgare*, grown under similar conditions, averaged 22.7 culms per plant, and F_1 plants of the cross *triuncialis* \times *vulgare* averaged 33.

In height these same greenhouse-grown plants averaged about 18 inches in *Aegilops ovata* and 22 inches in *A. triuncialis*. The height of the wheat plants varied with the variety, averaging about 30 inches for some varieties of *T. compactum*, and about 42 inches for certain varieties of *T. vulgare*, *T. turgidum*, and *T. polonicum*, although some of these latter reached a height of about 60 inches. The F_1 *ovata* \times *vulgare* plants averaged about 34 inches in height, and the F_1 *triuncialis* \times *vulgare* about 37 inches. For the first-named cross the intermediate growth habit of the hybrid plant as compared with the parents is shown in Figure 16.

In number of awns produced the F_1 of the *Aegilops ovata* \times *Triticum vulgare* cross is intermediate, as is evident in Figure 17. The *ovata* glume usually has four or five well-developed awns, while the glume of wheat has only a single awn or beak, which is often very short. The



FIG. 16.—Plants: A, *Triticum vulgare*; B, F_1 of *Aegilops ovata* \times *Triticum vulgare*; C, *Aegilops ovata*

F_1 of the cross illustrated has two long awns and several teeth. In the different hybrids there is considerable fluctuation in this character, but the F_1 is always rather definitely intermediate between the parents. The same condition exists in the F_1 of the cross between A.

triuncialis and common wheat, the glumes of which are shown in Figure 17. Here again the hybrid is rather definitely intermediate between the parents in development of awns.

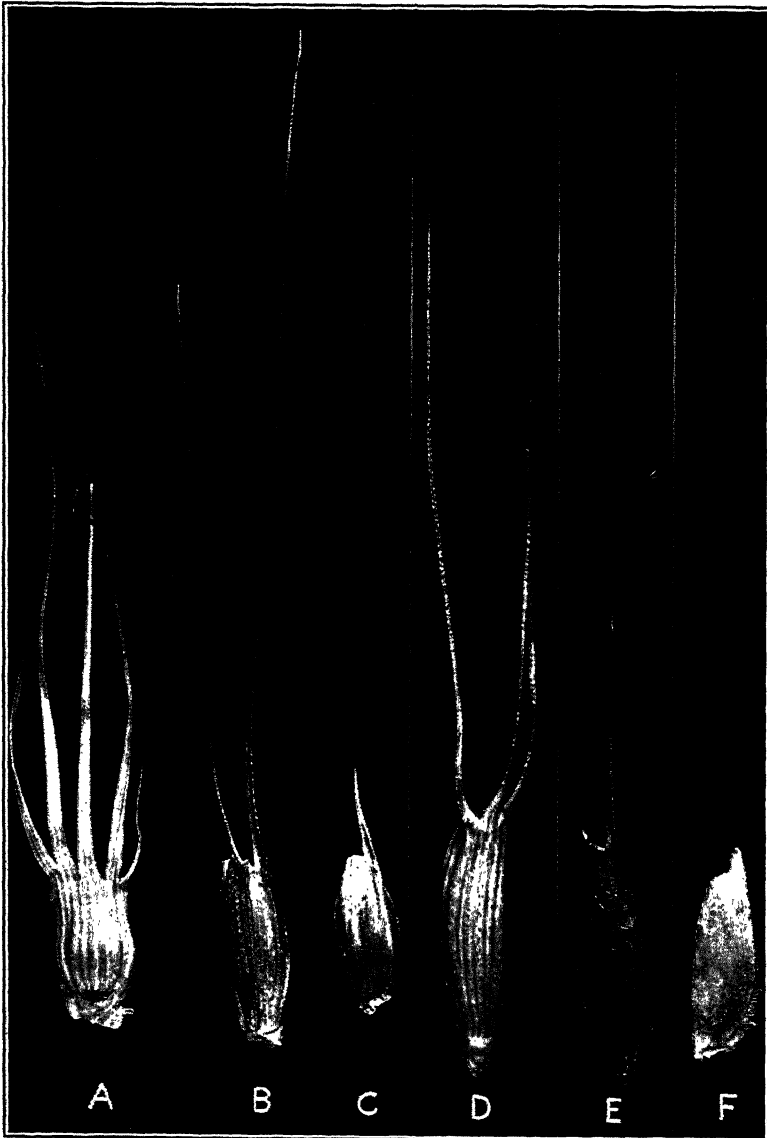


FIG. 17.—Glumes: A, *Aegilops ovata*; B, F_1 of *Aegilops ovata* ♀ × *Triticum vulgare* ♂; C, *Triticum vulgare*; D, *Aegilops triuncialis*; E, F_1 of *Aegilops triuncialis* ♀ × *Triticum vulgare* ♂; F, *Triticum vulgare*.

FERTILITY OF THE HYBRID PLANTS

The F_1 generation of *Aegilops* × *Triticum* hybrids generally has been found sterile, though Godron (9), Groenland (11), and Tschermak (32) present indisputable evidence that complete sterility does

not always occur. Godron believed the F_1 fertile only when backcrossed with wheat, but Tschermak suggests the possibility that some of the seeds in his F_1 plants were due to selfing. As no precautions appear to have been taken by Tschermak to prevent cross-pollination, his opinion is based on the fact that F_2 individuals which resembled the F_1 plants were self-fertile when pollination was controlled.

The present writers have had under observation more than 160 F_1 plants of crosses herein recorded between *Aegilops* and *Triticum*, grown under the following conditions: 7 fall-sown under field conditions; about 90 fall-sown in 6-inch pots in ordinary greenhouses; and about 60 likewise fall-sown in 6-inch pots, but in a greenhouse where the "length of day" was extended to about 16 hours by means of electric illumination. The plants grown under the lengthened-day conditions developed poorly, and were much inferior to those grown in ordinary greenhouses and in the field.

The flowers of the F_1 *Aegilops-Triticum* plants opened normally, and the anthers were extruded as in wheat. At the time of blooming these plants were examined frequently for any indications of fertility. Since self-fertility obviously can occur only when the anthers dehisce, careful scrutiny was maintained for this phenomenon. As shown in Table 4, natural dehiscence of anthers was observed on 16 plants, 14 of which were growing in the ordinary greenhouse and 2 in the field. On no plant in the lighted greenhouse did dehiscence occur, possibly on account of the inferior development of the plants. On the plants where dehiscence occurred the phenomena varied. In no case did all of the anthers on a single plant, or even all on a single spike, dehisce. Sometimes dehiscence occurred only on primary spikes, sometimes only on secondary spikes, but occasionally on both primary and secondary spikes. With the exception of those which dehiscid naturally, the anthers on all F_1 plants were more or less deficient in their proper function, and microscopic examination revealed but few, if any, pollen grains that were viable. All of the 16 plants on which anthers were observed to dehisce naturally, however, produced one or more seeds. In addition to these 16 plants there were 14 others belonging to the F_1 generation which produced seed naturally, but in which, on account of the limitations on observation, dehiscence was not observed. As shown in Table 4, these 30 plants of the two categories produced a total of 154 seeds. There is reason to believe that most, if not all, of these seeds were from self-pollination. Although natural crossing appears to occur but rarely in the greenhouses where these plants were grown, the seeds produced on plants setting seeds naturally, but in which dehiscence was not observed, may possibly have resulted from backcrossing, though this is considered improbable. Fortunately, the pollination of 7 of the 16 plants on which anthers were observed to dehisce was controlled by inclosing single heads in glassine bags or by isolation of individual plants in a greenhouse where no wheat or *Aegilops* was grown. As seeds were obtained under these conditions, there is no doubt that self-fertility may exist in the F_1 of *Aegilops-Triticum* hybrids, the so-called *A. triticoides*. Godron's (9) conclusion that *A. triticoides* is always self-sterile is not substantiated in these experiments, therefore, although self-sterility is by far the more common condition.

TABLE 4.—Numbers of seed-producing (self-fertile) F_1 plants of several *Aegilops-Triticum* hybrids in which the anthers were and were not observed to dehisce, and number of seeds produced in each case

Hybrid combination, and parental wheat variety	Seed-producing plants in which dehiscence was observed		Plants setting seed naturally, but in which dehiscence was not observed	
	Number of plants	Number of seeds produced	Number of plants	Number of seeds produced ^a
<i>A. ovata</i> × <i>T. dicoccum</i> :				
(Khapli).....	1	1	0	0
(Black Winter).....	3	6	1	11
<i>A. ovata</i> × <i>T. durum</i> :				
(Arnautka).....	1	12	0	0
(Kubanka).....	1	1	0	0
<i>A. ovata</i> × <i>T. vulgare</i> :				
(Ladoga).....	1	6	0	0
<i>A. triuncialis</i> × <i>T. dicoccum</i> :				
(Khapli).....	2	6	1	5
<i>A. triuncialis</i> × <i>T. dicoccoides</i>	2	3	1	2
<i>A. triuncialis</i> × <i>T. durum</i> :				
(Peliss).....	0	0	4	30
<i>A. triuncialis</i> × <i>T. turgidum</i> :				
(Alaska).....	2	44	0	1
<i>A. triuncialis</i> × <i>T. compactum</i> :				
(Dale Gloria).....	2	7	2	5
<i>A. triuncialis</i> × <i>T. spelta</i> :				
(White Bearded).....	0	0	3	7
<i>A. triuncialis</i> × <i>T. vulgare</i> :				
(Canadian Red).....	0	0	1	2
(Sonora).....	1	3	1	3

^a It is possible that some of these seeds were due to backcrossing, but this is considered improbable.

The only hybrids between *Aegilops ovata* and *Triticum* which showed self-fertility were those involving two varieties each of *T. dicoccum* and *T. durum*, and one variety of *T. vulgare*. The total percentage of self-fertility was very low. The 40 F_1 plants of the *ovata-vulgare* cross alone bore at least 15,000 flowers, but only 6 seeds were produced from self-pollinations. On the other hand, self-fertile F_1 plants were produced when *A. triuncialis* was crossed with *T. dicoccum*, *T. dicoccoides*, *T. compactum*, *T. turgidum*, and *T. vulgare*, and probably with *T. durum* and *T. spelta*, although, as already explained, dehiscence of anthers was not actually observed in these two latter crosses. The 49 plants of *triuncialis* and *Triticum* parentage produced 117 seeds when pollen was not applied artificially, while under the same circumstances the 97 plants of *ovata* and *Triticum* parentage produced only 37 seeds. Of the 117 seeds produced in the *triuncialis* crosses, however, 30 were produced in one cross, involving 4 plants of which Peliss durum wheat was one parent, and 44 seeds were from 2 plants of *A. triuncialis*-*T. turgidum* parentage. But one plant of the cross *ovata* × Black Winter emmer produced 11 seeds, and one plant of the cross *ovata* × Arnautka durum produced 12. So, while there is some evidence of greater general compatibility between *Triticum* and *triuncialis* than between *Triticum* and *ovata*, it is not conclusive in nature.

BACK CROSSES ON THE F_1

Numerous back crosses were made on the F_1 *Aegilops-Triticum* hybrids grown in 1923, pollen not only from the parental wheat species represented in the original cross, but also from other species being employed in many cases, as is shown in Table 5. Since no

selfed seeds had been obtained, and no anthers had been observed to dehisce on F_1 plants grown previous to 1923, the usual procedure of emasculation had been dispensed with in pollinating the F_1 with parental types. A few successful back crosses thus made in 1922 proved their hybrid origin by certain characters which they possessed. This method was again followed in 1923, and all artificial pollinations of the F_1 plants of that year, shown in Table 5, were made without emasculation. On account of the self-fertility, which first was exhibited in this year in certain plants, it may be that a few seeds listed in Table 5 as originating from back crosses with wheat or *Aegilops* species may actually have originated by selfing.

TABLE 5.—Number of flowers pollinated and seeds set on F_1 plants grown in 1923 from crosses between *Aegilops ovata*, *A. triuncialis*, and different species of *Triticum* when back crossed with parental and other species

Hybrid combination	T. vulgare		T. compactum		T. spelta		T. dicoccum		T. dicoccoides	
	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set
<i>A. ovata</i> ×										
<i>T. vulgare</i>	1,137	17			93	0	86	0		
<i>T. spelta</i>	214	12					16	0	16	0
<i>T. dicoccum</i>	136	2	18	0	16	0	94	0		
<i>T. turgidum</i>	446	12			32	0	62	0	28	0
<i>T. durum</i>	286	20			26	2	60	5	16	0
<i>T. polonicum</i>	70	1					30	0		
<i>T. monococcum</i>							14	0		
Total.....	2,289	64	18	0	167	2	362	5	60	0
<i>A. triuncialis</i> ×										
<i>T. vulgare</i>	984	16			62	0	82	0	122	0
<i>T. compactum</i>			92	1	46	3				
<i>T. spelta</i>	78	4			66	1				
<i>T. durum</i>	296	16			76	4	34	3	32	2
Total.....	1,358	36	92	1	250	8	116	3	154	2

Hybrid combination	T. turgidum		T. durum		T. monococcum		A. ovata		A. triuncialis	
	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set	Flowers pollinated	Seeds set
<i>A. ovata</i> ×										
<i>T. vulgare</i>			50	0			142	1		
<i>T. spelta</i>	18	1								
<i>T. dicoccum</i>							42	0		
<i>T. turgidum</i>				1			16	0		
<i>T. durum</i>			72	1			14	1		
<i>T. monococcum</i>			24	0	28	0				
Total.....	18	1	146	1	28	0	214	2		
<i>A. triuncialis</i> ×										
<i>T. vulgare</i>			18	0			20	0	124	0
<i>T. compactum</i>			44	1					46	0
<i>T. spelta</i>			40	3					90	0
<i>T. durum</i>			78	5						
Total.....			180	9			20	0	260	0

The crosses in which self-fertility was observed appear in Table 4, and the back crosses made appear in Table 5. The F_1 plants of the hybrids between *Aegilops ovata* and different varieties of *Triticum vulgare* (Table 1) were self-sterile, with the exception of the one in

which Ladoga was used as the *vulgare* parent, but 1.5 per cent of the flowers back crossed with *vulgare* pollen produced seeds. Back crosses with *vulgare* pollen were performed on 1,137 flowers borne by 10 F_1 plants from 8 of the crosses with different wheat varieties listed in Table 1. None of these plants produced many kernels when thus back crossed, but most of them were fertile to a small degree, a total of 17 kernels being produced. When back crosses were made on these F_1 plants with pollen of the *Aegilops* parent, *A. ovata*, only one kernel was produced by the 142 flowers pollinated. The pollen of *T. spelta*, *T. dicoccum*, and *T. durum* when used on these F_1 *ovata* \times *vulgare* plants did not result in the production of a single seed.

Back crossing with *Triticum vulgare* pollen also resulted in seed production in one or more plants of all of the remaining *ovata*-*Triticum* hybrids, except those in which *T. compactum* or *T. monococcum* was used, in which cases back crosses were not attempted. Back crosses with *T. turgidum* pollen resulted in seed production only in the *A. ovata* \times *T. spelta* hybrid. Seed was produced in the *A. ovata* \times *T. durum* hybrid when pollinated by *T. vulgare*, *T. spelta*, *T. dicoccum*, *T. durum*, and *A. ovata*. Other attempted back crosses on *A. ovata* \times *Triticum* hybrids were without result. Since a complete series of back crosses was not attempted, and conditions affecting those made were not fully comparable, the relative fertility of different hybrids and the effectiveness of pollens from different sources is not definitely established.

Back crosses on the hybrids between *Aegilops triuncialis* and *Triticum* were partially effective in each case when *vulgare* pollen was used, seed being produced by the F_1 of the *triuncialis* crosses with *vulgare*, *spelta*, and *durum*. The *triuncialis* \times *vulgare* hybrids produced seed from back crosses only when *vulgare* pollen was used and three plants of this hybrid were self-fertile. The *triuncialis* \times *compactum* hybrids produced seed from back crosses with *compactum*, *spelta*, and *durum*. The *triuncialis* \times *spelta* hybrids produced seed from back crosses with *vulgare*, *spelta*, and *durum*. The *triuncialis* \times *durum* hybrids were the most generally responsive to back crossing, seed being produced from pollinations by *vulgare*, *spelta*, *dicoccum*, *dicoccoides*, and *durum*, the only species used in back crossing. Pollen of *triuncialis* was not effective in back crosses on hybrids of which it was one of the parents.

Of the 3,088 F_1 *Aegilops ovata* \times *Triticum* flowers pollinated with wheat species, 73, or 2.4 per cent, set seed, while of the 2,150 F_1 *A. triuncialis* \times *Triticum* flowers similarly pollinated, 59, or 2.7 per cent, set seed. The two groups of hybrids thus appear to be about equally responsive to back crossing.

The low degree of self-fertility exhibited by these hybrids, and the increased but still low seed production resulting from back crossing, just cited, are in marked contrast to the results obtained in the original crosses. When *ovata* and *triuncialis* were crossed with different species of wheat as pollen parents, about 45 per cent of the pollinations were successful. It is plainly evident that the success attained in the original cross is no index of the fertility in the F_1 generation.

In this connection it should be noted that F_1 hybrids involving both *Aegilops ovata* and *A. triuncialis*, of which durum wheat was one parent, were the most generally fertile of all the hybrid com-

binations studied, on back crossing with the different wheat species. The *A. ovata* × *Triticum durum* hybrid produced seed when back crossed with 4 different *Triticum* species and also with *ovata*, and the *triuncialis* × *durum* hybrid produced seed when back crossed with 5 different *Triticum* species. Self-fertility also was present in certain plants of each group of these *durum* hybrids.

The reason is not evident for this apparently greater compatibility between *Triticum durum* and the two species of *Aegilops* used than was found in hybrids with other species of *Triticum*. The chromosome number in *durum* is 14 (haploid), the same as in *ovata*, but *turgidum*, *dicoccum*, and *polonicum* also have 14 chromosomes, so the chromosome number does not appear to be the decisive factor. Backcrosses with wheats with 14 and 21 chromosomes also were about equally effective in seed production. The F_1 plants of these *durum* hybrids made a vigorous growth, but did not exceed certain other hybrids in this respect. It appears, then, that there is a relatively greater degree of compatibility between certain *durum* wheats and the species of *Aegilops* used, than between the other wheat species and these *Aegilops* species, for which no satisfactory explanation can be given.

A STUDY OF THE SO-CALLED AEGILOPS SPELTAEFORMIS

The plants obtained as a result of back crossing the F_1 of *Aegilops-Triticum* hybrids with pollen of wheat sometimes have been known as *A. speltaeformis*. Jordan (14), who first proposed this name, believed that such plants formed a natural species.

The present hybridization studies have resulted in the production of many such plants, some of which already have matured. Data obtained on eight plants of this kind are given in Table 6 and spikes from three plants are shown in Figure 18. Seven of these plants were developed by back crossing the F_1 *Aegilops ovata* × *Triticum vulgare* hybrid with *T. vulgare* pollen, and one by similar back crossing with *T. spelta* pollen.

TABLE 6.—Data on height, dehiscence of anthers, and number and percentage of seed produced on eight plants of *Aegilops-Triticum* parentage by selfing and by pollination with wheat

Parentage of plants	Height of plant	Anthers dehiscing	Number of flowers selfed	Seed set		Number of flowers pollinated with wheat	Seed set	
				Number	Per cent		Number	Per cent
1. (<i>A. ovata</i> × <i>T. vulgare</i>) × <i>T. vulgare</i> .	36	All.....	262	1	0.38	22	1	4.55
2. Do.....	34	All.....	188	17	9.04	56	9	16.07
3. Do.....	30	None.....	222	0	0.00	168	9	5.36
4. Do.....	26	Part.....	200	0	0.00	30	5	16.67
5. Do.....	33	Part.....	135	1	.5	40	0	0.00
6. Do.....	34	All.....	204	0	0.00	124	10	8.06
7. Do.....	30	None.....	94	0	0.00	72	1	1.39
8. (<i>A. ovata</i> × <i>T. vulgare</i>) × <i>T. spelta</i> .	33	Part.....	90	3	3.33	142	43	30.28
Total.....			1,385	22		654	78	
Average.....	32				1.59			11.93

These plants varied in height from 26 to 36 inches, averaging 32 inches. The anthers dehiscenced fully in three of these plants, partly in three plants, and not at all in two plants. Four of the plants produced from 1 to 17 seeds by self-pollination, the percentage of self-



FIG. 18.—A, B, C, Spikes of the so-called *Aegilops speltaeformis*. (See text)

fertility varying from 0.8 to 9.04 per cent. Seven of the plants produced from 1 to 43 seeds when pollinated with wheat, the percentage of the flowers setting seed varying from 1.39 to 30.28 per cent of those pollinated.

These results are comparable to those of Fabre and Godron who obtained plants of the so-called *Aegilops speltaeformis* which apparently were self-fertile.

SUMMARY

This paper reports the results of an extensive series of hybridizations of *Aegilops ovata* and *A. triuncialis* with *Triticum monococcum*, *T. dicoccum*, *T. dicoccoides*, *T. durum*, *T. polonicum*, *T. turgidum*, *T. compactum*, *T. spelta*, and *T. vulgare*, and also with *Secale cereale*. *A. ventricosa* also was used in a few cases.

Of the flowers of *Aegilops ovata* pollinated by *Triticum*, 44.6 per cent produced seed; and of *A. triuncialis* flowers so pollinated, 45.5 per cent produced seed. Reciprocal crosses resulted in 3.1 per cent of fertility with *A. ovata* pollen on *Triticum* species, and 10.4 per cent with *A. triuncialis* pollen. When *Secale cereale* pollen was used on *A. ovata* and *A. triuncialis* flowers the percentages of fertility were 28.3 per cent and 50 per cent, respectively. Seeds obtained in *Aegilops*-*Secale* crosses were poorly developed and germinated very poorly, and the plants obtained have been entirely sterile.

F_1 plants were matured from crosses between *Aegilops ovata* and one or more varieties of each species (or subspecies) of *Triticum* named, and also *Secale cereale*; the same is true for *A. triuncialis*, except in the cross with *T. monococcum*. Although there is a general resemblance of the F_1 plants to the *Triticum* parent, *Aegilops* characters predominate.

Self-fertility was shown to exist in the F_1 of *Aegilops*-*Triticum* hybrids, although self-sterility is by far the most common condition.

Numerous backcrosses were made on the F_1 plants, pollen from the parental and other species being used in many cases. Of the *Aegilops ovata*-*Triticum* flowers pollinated by *Triticum* species, 2.4 per cent produced seed; of the *A. triuncialis*-*Triticum* flowers, 2.7 per cent. Two seeds were produced in 214 F_1 *A. ovata*-*Triticum* flowers pollinated by *A. ovata* pollen. *A. ovata* and *A. triuncialis* pollen did not produce seed when used on F_1 *A. triuncialis*-*Triticum* flowers.

Hybrids in which *Triticum durum* was one parent were the most generally fertile on backcrossing, and were at least equally self-fertile in comparison with other hybrid combinations.

Plants obtained by backcrossing the F_1 of *Aegilops*-*Triticum* hybrids with pollen of wheat, often called *A. speltaeformis* by earlier writers, were partially self-fertile.

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ANATOMY OF THE VEGETATIVE ORGANS OF THE SUGAR BEET¹

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INTRODUCTION

The first critical description of the structure of the sugar beet was published by van Tieghem (15).² He traced the ontogeny of the primary tissues and the mode of origin of the supernumerary cambiums and the endodermis, deriving both these tissues from the pericycle.

Later investigators—de Bary (1), Morot (7, p. 241), Fron (5), and Strasburger (14)—mainly substantiated the work of van Tieghem.

As the early investigations were chiefly concerned with the origin and nature of the anomalous growth of the beet, they were naturally limited in their scope. An insight into the organization of the plant in its entirety was given by Droysen (4) and de Vries (16), and the work of the latter has remained a classic to the present day.

As the sugar beet gained in importance as an economic plant, a series of both technical and popular papers appeared dealing with certain features of the beet structure. The popular articles were largely by Briem (2), and were intended to give information to beet growers. Of the purely scientific papers, the account of Wiesner (17) deserves first mention. This contains an accurate description and classification of the different tissues of the beet, with special emphasis on the composition of the walls of the storage parenchyma. An understanding of the localization of the sugar in the different tissues was obtained through the researches of Peklo (8) and Colin and Grandsire (3).

Numerous attempts were made to correlate anatomical structure and sugar content, in the hope of arriving at better methods of selection in breeding for higher sugar content. The futility of these attempts is shown by the contradictory evidence obtained by Schindler (11), Briem (2), Schneider (12), Peklo (8), Geschwind (6), and numerous others.

Certain features of the anatomical structure have been critically reinvestigated in the last two decades. Through the work of Plaut (9), Rüggeberg (10), and Seeliger (13) there is now a correct understanding of the structure and distribution of the endodermis, the fate of the primary cortex, and the development of the primary and secondary tissues of the beet. The detailed and painstaking investigations of Seeliger have led to a modification of van Tieghem's concept of the origin of the supernumerary cambiums which had been taken over unreservedly by other investigators.

The object of the present paper is to give a compendium in the English language of the present knowledge of the structure of the

¹ Received for publication Dec. 11, 1925; issued July, 1926.

² Reference is made by number (italic) to "Literature cited," p. 175.

sugar beet. The results of earlier investigations, carried on by different writers and at different times, were in need of testing; and further investigations were necessary in order that there might be obtained a picture of the inner organization of the beet which would be in harmony with present ideas. Comparative studies, so necessary for the proper evaluation of the normal anatomical picture, were limited to imperative requirements, but they will be extended by subsequent investigations.

MATERIAL AND METHODS

The material was obtained partly from seedlings grown in the greenhouse, and partly from beets growing in the field in Colorado. The material was fixed with Flemming's medium solution and stained: (a) Delafield's haematoxylin and safranin, (b) Haidenhain's haematoxylin and safranin, (c) aniline blue and safranin. Free-hand sections were examined in chloriodide of zinc or stained with phloroglucin and hydrochloric acid counterstained with chloriodide of zinc. Cork membranes were stained with Sudan glycerin.

All drawings are from photomicrographs, which were taken on Wratten M plates with B 58 and E 22 filters used singly and in combination.

GROSS MORPHOLOGY

Beta vulgaris L. is a herbaceous dicotyledon, a member of the Chenopodiaceae. It normally completes its vegetative cycle in two years. During the first year it develops a large succulent taproot in which much reserve food is stored, and during the second year it produces flowers and fruits.

The mature beet is an elongated pear-shaped body composed morphologically of three regions—the crown, the neck, and the root. The crown is the broadened, somewhat cone-shaped apex. It bears a tuft of large succulent leaves and leaf bases. Adjoining it is the neck, a smooth narrow zone which is the broadest part of the beet and which constitutes ontogenetically the thickened hypocotyl. The root region, which forms by far the bulk of the beet tissues, is cone-shaped and terminates in a slender taproot. It is flattened on two sides, and often is more or less markedly grooved. The two depressions extend vertically downward or form a shallow spiral, and contain the lateral rootlets indistinctly arranged in two double rows. The surface of the beet is covered by a thin cork layer yellowish-white in color except on the aerial parts and at places of injury.

A well-formed beet has only one taproot. Occasionally the taproot branches and forms a number of thick stubby roots. The lateral roots are filiform, and originate from the two-arch xylem plate or from more peripheral rings of growth.

The leaves are arranged on the crown in a close spiral with the divergence of 5/13. The cotyledons, however, are arranged in opposite and decussating pairs. The lamina of the leaf is elongate triangular with rounded tip and undulate margin; the base is cordate and decurrent on the petiole, the latter being triangular in cross section and more or less flattened at the base. The venation is of the netted type. Lateral branches arise from a strongly developed midrib. These run obliquely outward, but before reaching the periphery they bend abruptly, run parallel with the surface, and

unite with the terminal ends of other lateral veins. The smaller veins anastomose freely, with the ultimate branches ending blindly in the parenchyma of the intercostal fields (fig. 1, A, B; fig. 10, B).

Unlike most cultivated plants, the beet shows a striking lack of uniformity in foliage characters. The most diverse types may be found growing side by side; plants with erect or flat foliage; short or long petioles, with lamina triangular or oblong; and straight or wavy margin and smooth or crinkly surfaces. There is also a great variation in the texture and thickness of the leaves as well as in the color, which may be either a dark green or a light olive, with numerous gradations between the two. When this paper was written investigations were underway with the object of isolating distinct foliage types in the hope of getting them sufficiently pure to breed true to form.

ANATOMY

STRUCTURE OF THE MATURE BEET

A median horizontal section through a mature beet shows a number of annular zones or rings of growth which are more or less equidistant, except near the periphery where they are very close together (fig. 2, A, B). Often, instead of a complete ring, smaller or larger segments of rings appear here and there; these are connected by their margin to the next inner ring. The center of the cross section is occupied by a two-lobed, more or less star-shaped core (fig. 3) from which radiate horizontal strands of vascular tissue—the root traces.

Each annular zone of growth is made up of a narrow ring of vascular tissue and a broad band of storage parenchyma. The ring of vascular tissue is composed of numerous collateral bundles separated from one another by medullary-ray tissue of varying width (fig. 4). In median longitudinal section the bundles of an individual ring are seen to anastomose tangentially, and between the bundles of successive rings there are obliquely descending radial connecting bundles. In the narrow tapering zone of the beet the number of annular rings decreases as the rings gradually unite with one another. A union of the rings is also effected in the apical leaf-bearing end, but here it is brought about by the passing out of the leaf traces. Each leaf is supplied with a number of traces of different degrees of development. The central trace extends horizontally through the complete radius of the cross section; the lateral traces do not extend so far, or they may even remain at the periphery. Owing to this arrangement, as pointed out by de Vries (16), the traces of one and the same leaf attach themselves to different annular rings, and, since they also tend to anastomose with one another, the relationship between all the leaves and the annular rings becomes very intimate.

The neck of the beet has, on the whole, root structure, except that in the upper region the central core opens up and incloses pith. As the neck passes into the crown this pith widens and frequently becomes hollow.

The composition of the vascular tissue is more or less alike for all bundles, with the exception of the central core and the peripheral region. The latter, however, differs only in that its bundles are immature, often represented only by undifferentiated cambium. The central core, on the other hand, exhibits a fundamental difference in that it contains both primary and secondary tissues. Its composition will be described in detail under development.

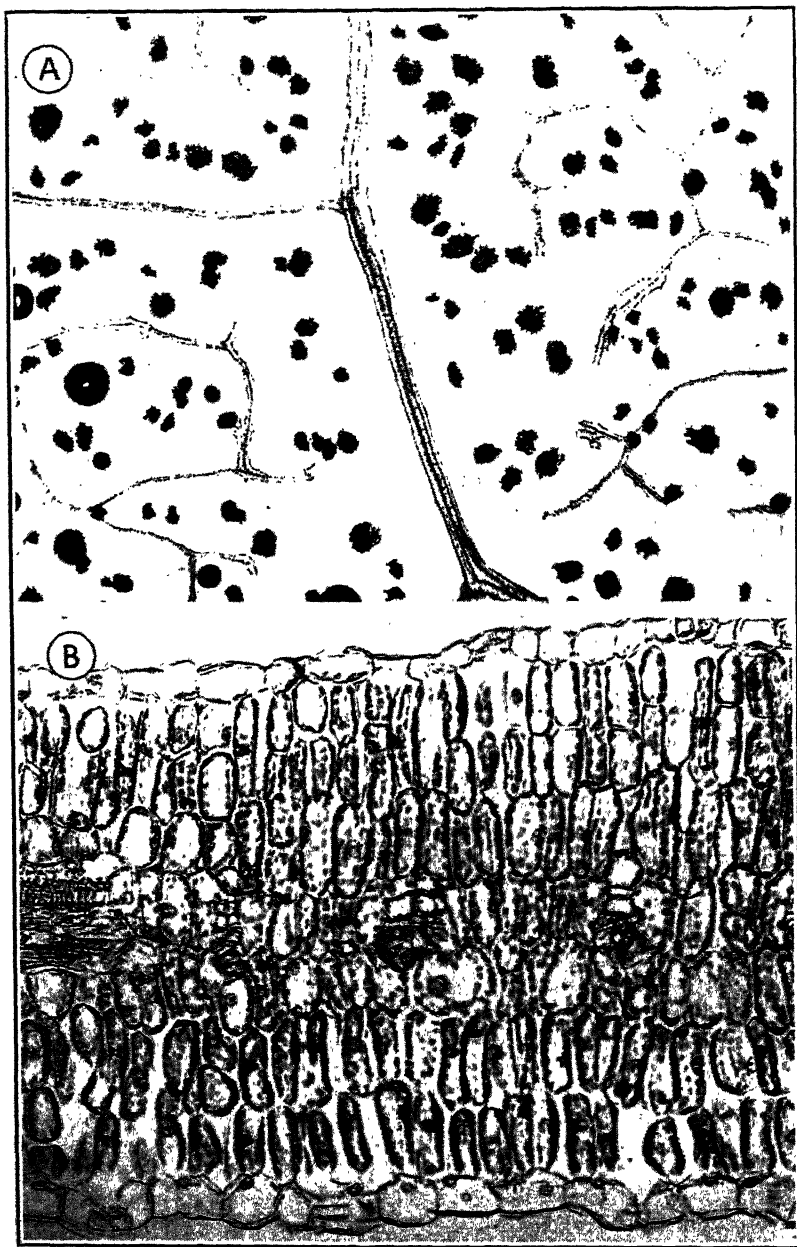


FIG. 1.—A. Part of beet leaf bleached to show venation. Note appearance of terminal veins ending blindly in the parenchyma. Dark spots are aggregates of calcium oxalate located in certain cells of the central mesophyll. $\times 66$. B. Cross section through a thick leaf. Note that all of the mesophyll consists of palisade cells. $\times 272$

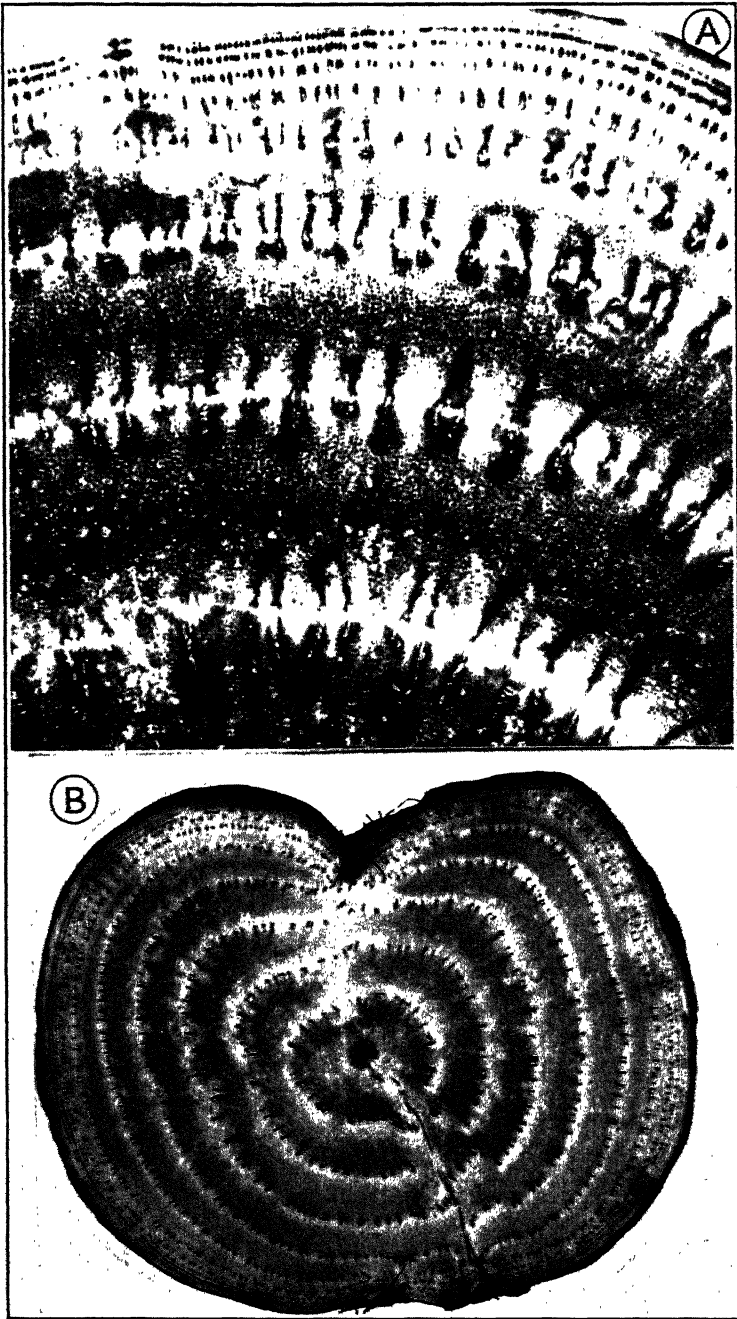


FIG. 2.—A. Partial cross section of a mature beet. $\times 475$. B. Cross section of mature beet, natural size. Section treated with phloroglucin-hydrochloric acid to bring out the vascular bundles

The bundles of the mature rings are widest in the region of the cambium and taper gradually toward the phloem and xylem pole. This gives them the appearance of a double wedge.

The xylem is formed of a radial row of vessels with some lateral thick-walled tracheids and wood parenchyma (fig. 5, A, B). Fibers are found in only the oldest rings of the hypocotyl. The vessels are reticulate; the large ones are porous and have short articulations; the cross walls are strictly transverse but often sloping. The small vessels are more like tracheids, much pointed but porous. Typical tracheids are found only occasionally. The wood parenchyma is elongated and pointed, sometimes cross septate. The walls are perforated by numerous pits; the latter are round, sometimes lathyrate. The fibers, whenever they occur, are elongated and intertwine with their tips. In cross section the fibers are round or prismatic. The cells surrounding the xylem are thick walled and collenchymatous and lack intercellular spaces.

The phloem is composed of sieve tubes, companion cells, and phloem parenchyma (fig. 6, A, B; fig. 7, A). The sieve tubes have terminal as well as lateral sieve plates (fig. 7, B), which soon become covered with callus. The companion cells are of the same length as the sieve tubes, but sometimes they become cross septate. The phloem parenchyma cells are elongated and pointed, often also cross septate. There are numerous cells transitional to the typical storage parenchyma. The walls of the phloem parenchyma cells are strongly pitted, and the corners of all three types of phloem cells are more or less collenchymatously thickened.

The first-formed phloem groups of a bundle are seen as small obliterated areas recognizable only by their staining reaction. They occur commonly at some distance from the bundle due to enlargement and division in the phloem parenchyma.

Between the different bundles of a ring lies a narrow band of intermediate tissue, which, with reference to the bundle itself, is like a normal medullary ray. The parenchyma of these rays consists of radially elongated cells which undergo tangential divisions as the annular zones increase in diameter. Secondary medullary rays frequently develop inside the vascular bundles, causing a forking of the latter.

The concentric rings of vascular tissue are separated from one another by broad bands of storage parenchyma. The cells are large and almost spherical, the walls thin and extensively pitted. The outer and inner peripheries of this interzonal parenchyma contain vascular elements in addition; the outer periphery scattered xylem cells, the inner periphery obliterated phloem.

The lateral roots of the beet are very thin and are covered for a considerable distance with root hairs. The anatomical picture of root structure in these roots differs from that of the thickened taproot in a preponderance of xylem cells, especially vessels (fig. 8). Ray parenchyma is practically wanting. Often the primary xylem plate is triarch instead of diarch, as is always the case in the taproot.

STRUCTURE OF THE LEAF

As seen in cross section, the vascular tissue of the petiole (fig. 9, A, B) forms a triangle which gradually widens basipetally. The number of bundles varies with the size and development of the leaf.

As the petiole merges into the midrib the number of bundles decreases, in that progressively more and more bundles fuse with one another. The large bundles of the petiole run separately from the base of the petiole to the insertion point of the lamina, while the smaller ones anastomose freely and form a network of wide meshes. The bundles are surrounded on all sides by cortical tissue which merges into col-

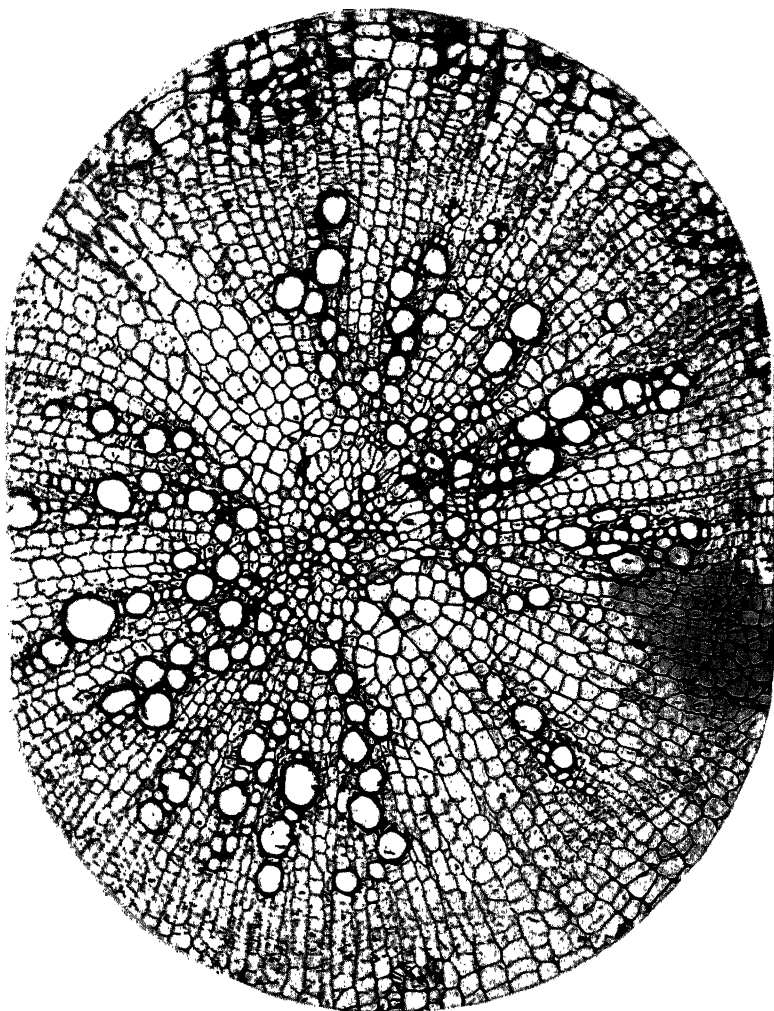


FIG. 3.—Central core of a young, actively growing beet. $\times 118$

lenchyma just beneath the epidermis. The extent and distribution of the collenchyma can best be seen in Figure 9, A. This tissue forms a continuous band near the center of the abaxial surface and is otherwise limited to the projecting ridges. In the midrib it forms a continuous layer on both upper and lower surface.

The epidermis of the petiole is made up of elongated rectangular cells where it covers the collenchyma, and of somewhat elongated,

more or less polygonal, cells where it abuts directly on cortical parenchyma. The epidermis contains numerous stomates (fig. 10, A), except in the regions of collenchyma development. The cells of the

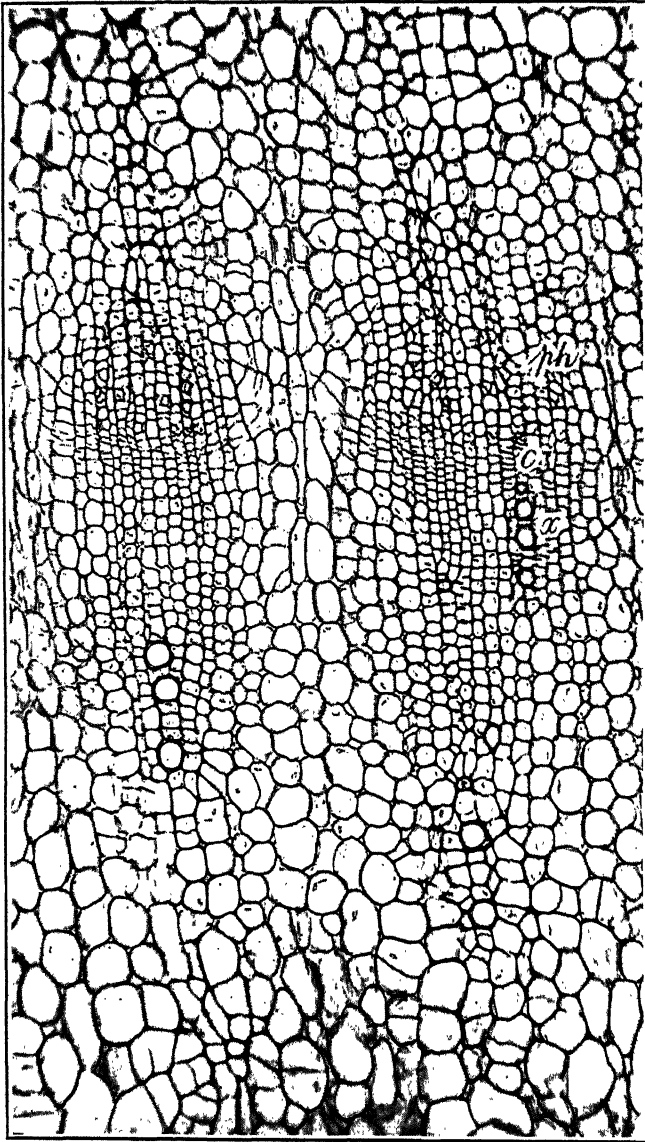


FIG. 4.—Vascular bundles from the outer zone of mature beet. *ph*, phloem; *c*, cambium; *x*, xylem, $\times 100$

petiolar cortex are large, more or less barrel shaped, and are separated by large intercellular spaces. The peripheral cells contain chlorophyll; others, more or less scattered throughout the tissue of the petiole, contain crystal sand.

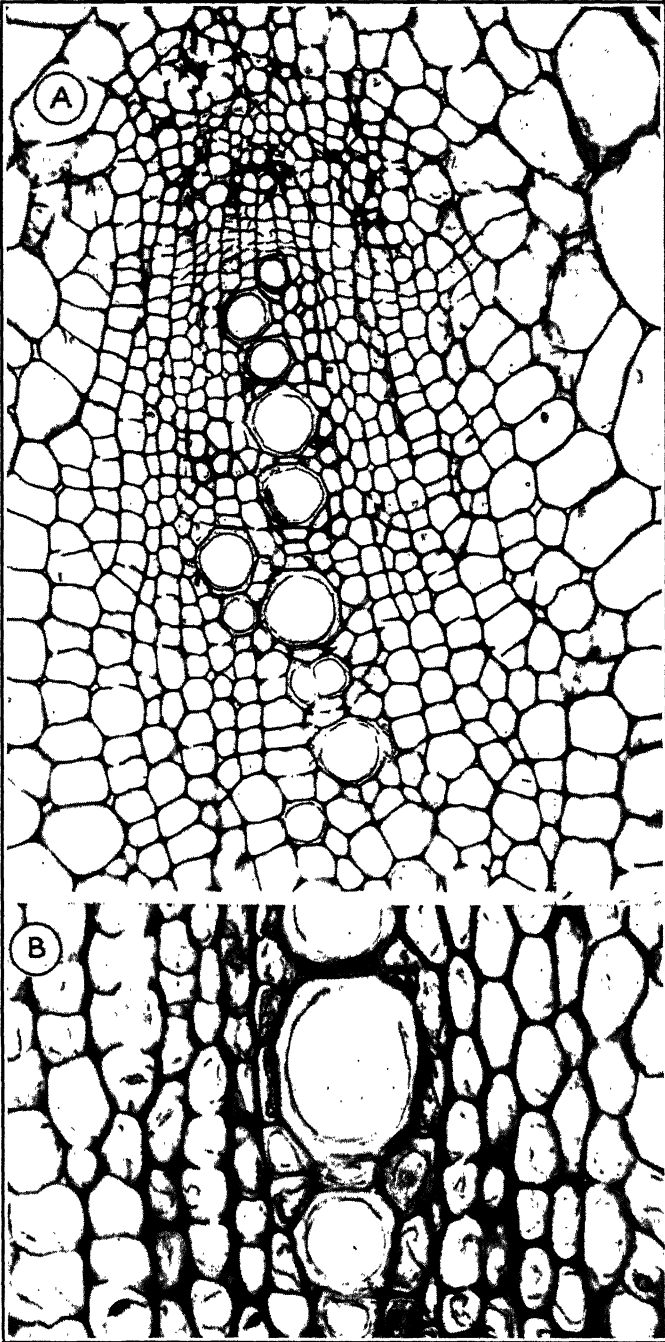


FIG. 5.—A. Typical bundle from mature beet. The sieve tubes show cellus deposit and partial degeneration. $\times 170$. B. Partial cross section of mature bundle. The parenchyma cells next to the xylem are thick walled and are spoken of as "sugar-sheath." $\times 500$

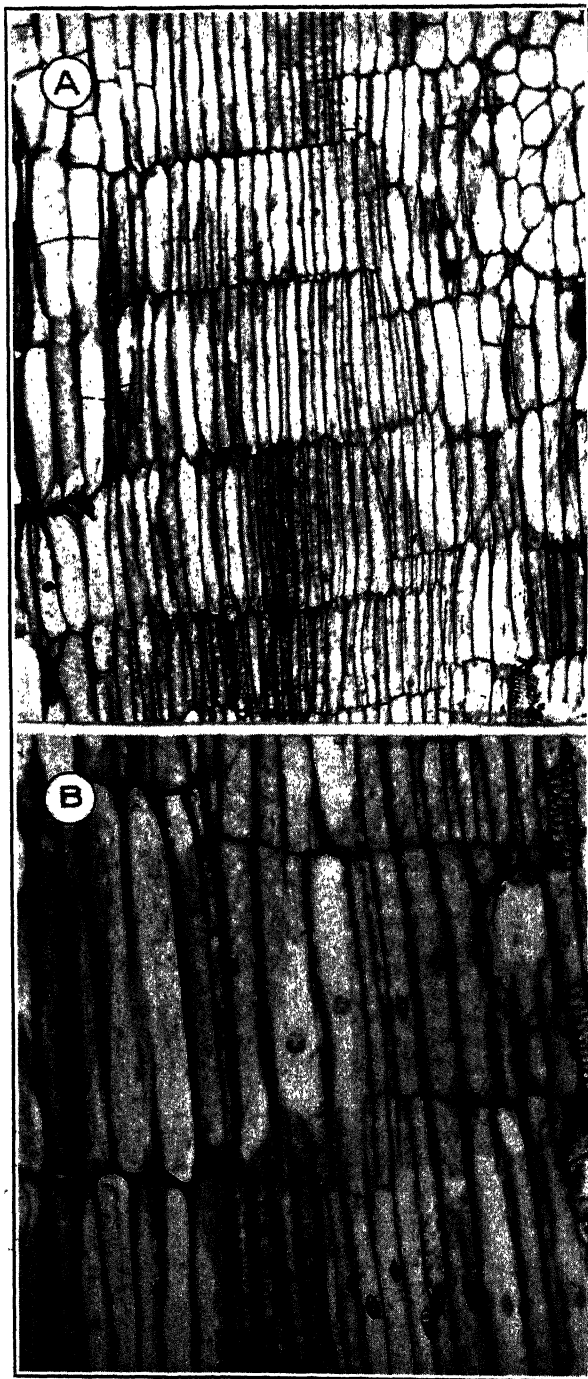


FIG. 6.—A. Radial section of phloem and cambium of actively growing ring. $\times 100$. B. Radial section of phloem and cambium. $\times 260$

The histological composition of the vascular tissue is that of typical collateral bundles. The phloem forms a narrow zone composed chiefly of sieve tubes with their companion cells and some phloem parenchyma. Above the phloem is a large sclerenchyma cap (fig. 11). A much less developed cap consisting of elongated thick-walled cells is found on the xylem pole of the bundle. Adjoining the latter is the protoxylem, interspersed with thin-walled fibers. The secondary wood forms a thick layer composed of numerous wide and narrow vessels and thick-walled fibers. Between xylem and phloem is the cambium formed of regular rectangular cells.

The epidermis of the lamina is unusual in being similar on both surfaces. The upper surface has irregular polygonal cells with tortuous walls (fig. 10, A). Toward the apex the cells become smaller. Here and there are small polygonal cells, the remains of young ephemeral hairs. In certain types of beets the mature leaves have very long, multicellular hairs, especially along the veins. Such leaves were sent to the writer from Rocky Ford, Colo., by A. W. Skuderna, who finds associated with this hairiness a high resistance to the leaf-spot disease. The cells of the lower epidermis are slightly more

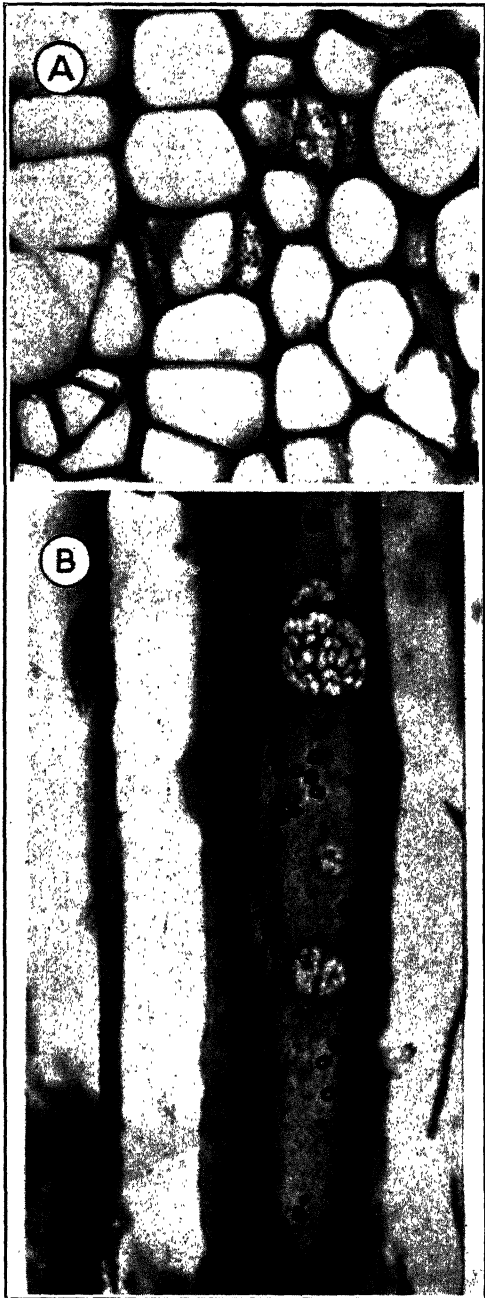


FIG. 7.—A. Mature phloem; the companion cells show granular content. $\times 1,000$. B. Mature sieve tube with lateral sieve plates. $\times 1,000$

irregular and the walls more tortuous. The stomates are of a simple type. The pores are surrounded by a pair of specialized guard cells which contain numerous chloroplasts. There are no accessory cells.

Stomates are found on both upper and lower surfaces (fig. 10, C), but are more numerous on the lower. De Vries (16) found on the upper surface an average of 91, and on the lower an average of 144

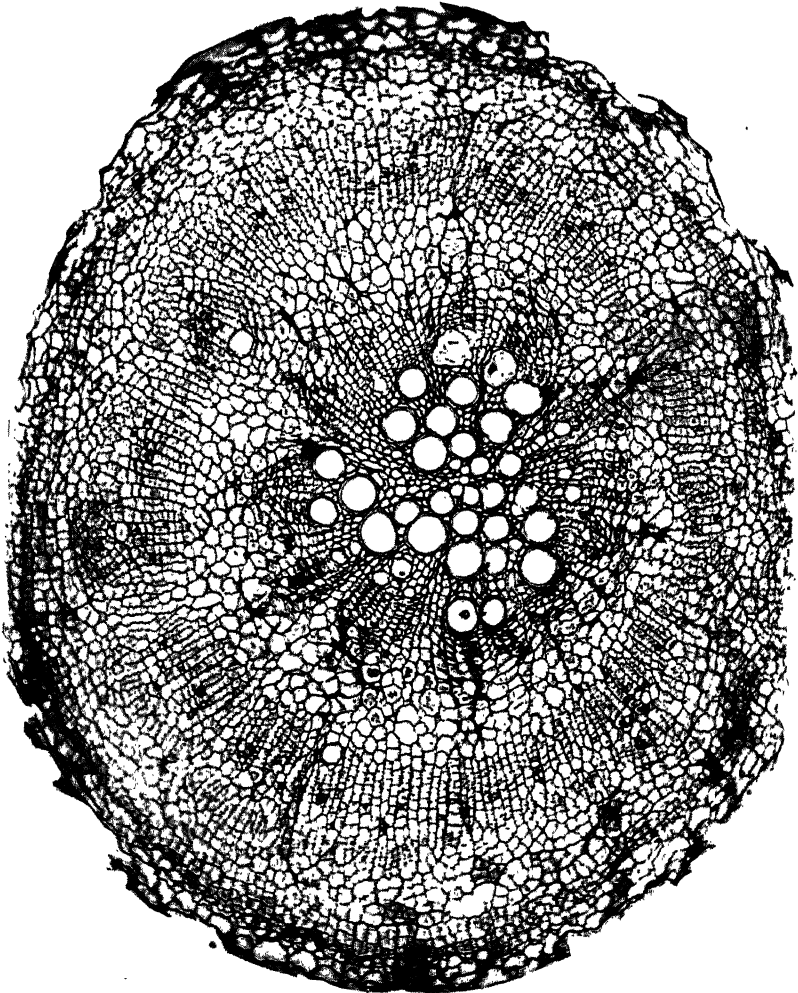


FIG. 8.—Cross section through tip of taproot. $\times 77$

per square millimeter. Droysen (4) found 114 and 162, respectively.

In determining the number of stomates only the degree of maturity of a leaf appears to be a factor. The size of the leaf as well as the different local areas of the lamina—apex, base, margin, middle—play a lesser rôle. It was found that the regional distribution shows as great variation as the fluctuation within the local areas themselves. Young leaves have naturally the largest number of stomates; as many

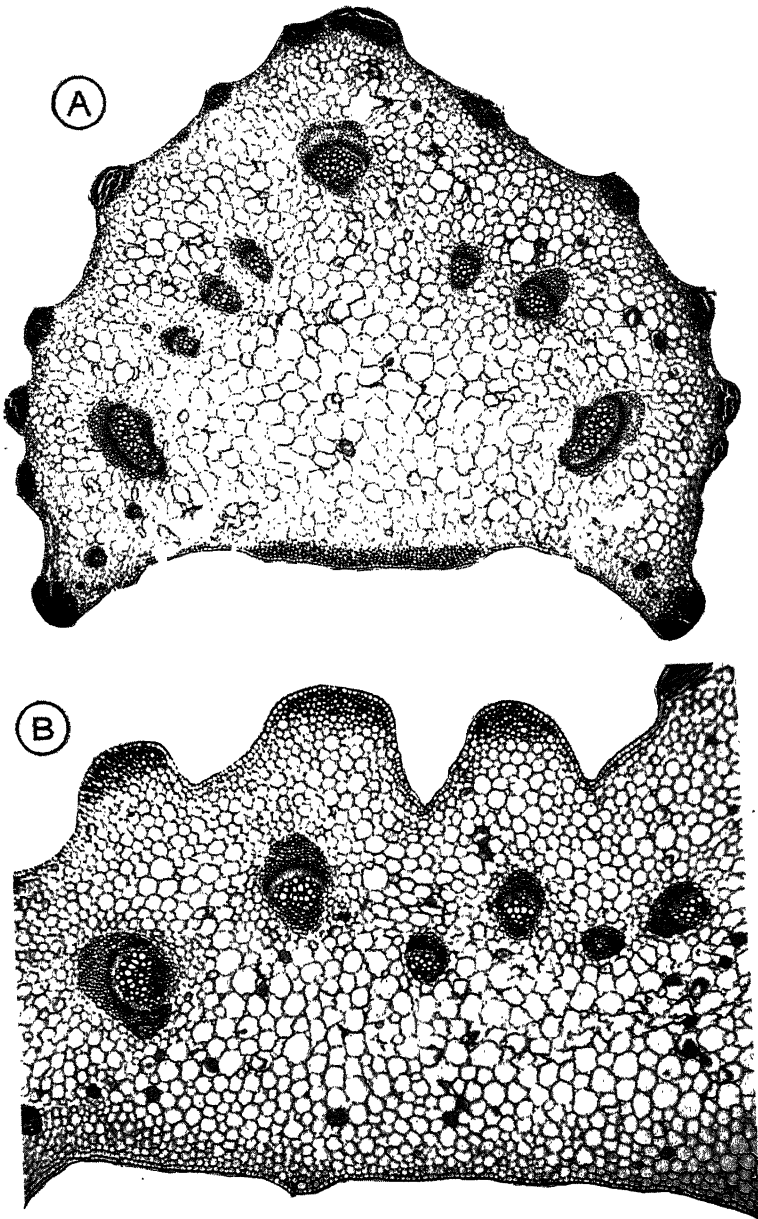


FIG. 9.—A. Cross section of petiole near the lamina. $\times 18$. B. Partial cross section of basal petiole. $\times 28$

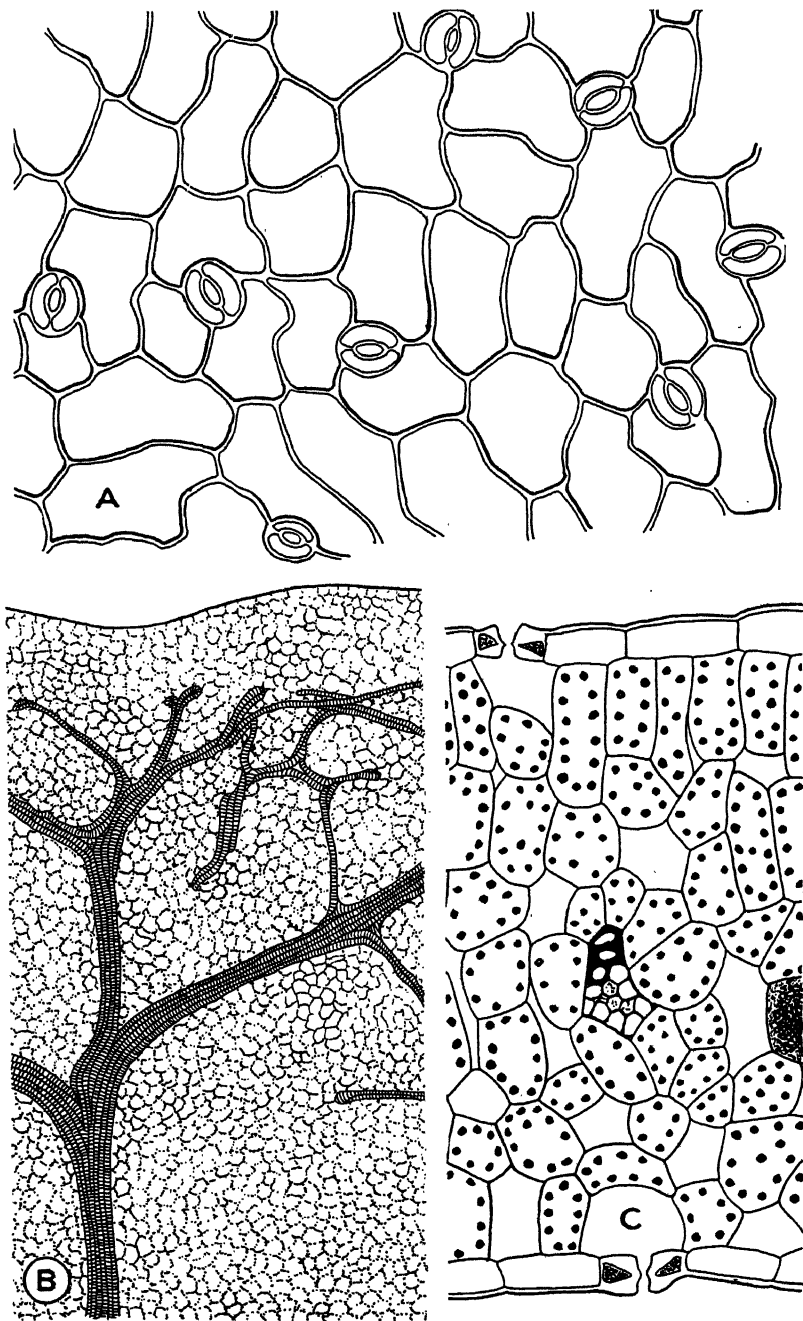


FIG. 10.—A. Upper epidermis, with stomates of mature leaf. $\times 296$. B. Margin of leaf with terminal veins. $\times 90$. C. Cross section of mature leaf [diagrammatic]. $\times 240$

as 300 and more have been counted per square millimeter. These large counts are due to the fact that the epidermal cells are very small and that the stomates have not expanded to their full size. In seemingly fully developed, though still young leaves, the number

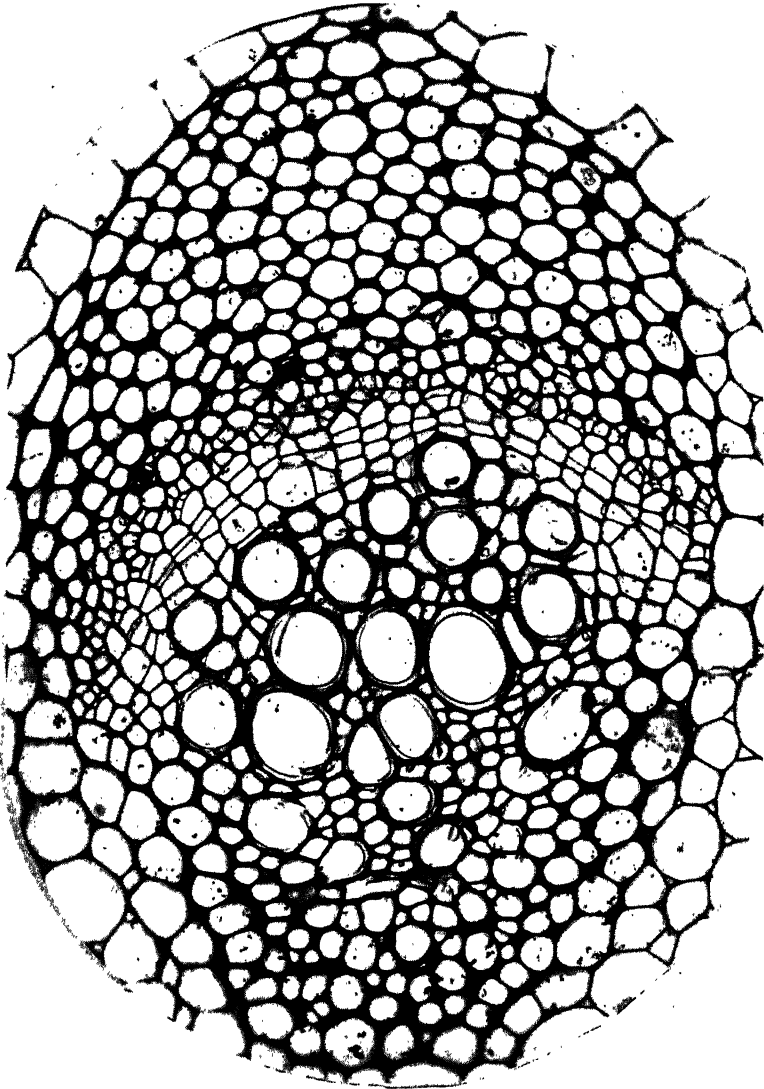


FIG. 11.—Cross section of petiolar bundle. $\times 303$

is still often quite high, with a maximum count of 240 on the lower surface. In fully matured leaves the number is less, but shows a great deal of fluctuation in the different foliage types. Table 1 gives the number of stomates on upper and lower surfaces of leaves of different color, size, and thickness.

TABLE 1.—*Color, size, and thickness of leaves, and number of stomates*

Color and size of leaf	Thickness of leaf	Number of stomates per square millimeter	
		Upper surface	Lower surface
	<i>Mm.</i>		
Light green; medium.....	0.32	104	122
Light green; large.....	.48	100	111
Medium green; medium.....	.35	130	145
Light green.....	.58	74	97
Dark green; large.....	.48	75	90
Dark green; very large.....	.56	60	59
Dark green; medium.....	.28	73	79
Medium green.....	.41	57	76
Light green.....	.36	90	114
Dark green.....	.45	76	119
Light green.....	.41	77	99
Light green.....	.40	138	167
Dark green.....	.72	88	111
Light green.....	.64	73	113
Dark green.....	.36	103	116
Dark green.....	.45	69	75

According to de Vries (16) and Droysen (4), the size of the stomates on the upper surface is 23 by 32 microns, while the size of those on the lower surface is a trifle smaller. Although these figures represent an acceptable mean, there is nevertheless quite a fluctuation in size in the different parts of a leaf and in different foliage types. Very old leaves have very large stomates, sometimes as long as 45 microns.

The mesophyll of the leaf is formed of parenchymatous tissue, the cells of which contain chloroplasts (fig. 10, C) and occasionally crystal sand. It is normally indistinctly divided into palisade tissue and spongy parenchyma. The palisade tissue is made up of small, more or less cylindrical cells; the cells of the spongy parenchyma are slightly larger and roundish in form.

The number of cell layers in a cross section of a leaf is practically constant, even though the thickness of the leaf varies greatly. In very thick leaves all cells are elongated into a uniform palisade tissue (fig. 1, B), whereas in very thin leaves typical palisade cells are altogether absent and the entire mesophyll consists of very short, roundish cells.

ONTOGENY

Microscopically, the young seedling shows three regions: Root, hypocotyl, and cotyledons. The junction between hypocotyl and root is indicated by an abrupt tapering of the axis and the appearance of lateral rootlets. In very young seedlings this demarcation is less distinct. Seeliger (13) includes in the root the region from the root-cap to the piliferous zone, while the hypocotyl extends from the piliferous zone to the insertion point of the cotyledons.

The cotyledons are elongate elliptical, with the lamina narrowing at the base to form a short petiole. The anatomical structure is very simple. The tissue of the lamina is indistinctly divided into chlorophyll-bearing palisade cells and spongy parenchyma. The

latter contains numerous calcium oxalate cells. The epidermis is simple, and is composed of irregular polygonal cells with somewhat tortuous walls. There are numerous stomates distributed equally over the lower and upper surfaces. In the region of the petiole the epidermal cells become elongated, while the stomates become fewer and finally disappear. The conducting tissue is represented by fine collateral bundles which run longitudinally between spongy parenchyma and palisade tissue.

Both root and hypocotyl are terete. The center is occupied by a thin strand of primary vascular tissue, which is inclosed by a cortex and bounded at the periphery by a single-layered epidermis. The epidermis in the apical root region is specialized, in that many of its cells are elongated to form hairs. The epidermis of the hypocotyl is cuticularized and, like the older root zone, is devoid of hairs.

The cortex is made up of three to seven rows of elongated barrel-shaped cells which are separated from one another by large intercellular spaces (fig. 12). The innermost layer of the cortex contains smaller and more regular cells; some of which contain crystal sand. Cortex and vascular tissues are separated by an endodermis. The cells composing this layer are four-sided and regular, and there are no intercellular spaces among them (fig. 12, D). The root tip, with the exception of the first few millimeters, has a primary endodermis which is characterized by the Casparian strips along the radial walls. Plaut (9) made a special study of the development of the endodermis of the sugar beet, and his findings were substantiated by this investigation. The primary endodermis extends axially over a distance of 3 centimeters, when it becomes secondary, which state is characterized by the development of a suberin lamella over the entire surface. The cells of the endodermis which lie opposite the protoxylem points pass into the secondary state later than the cells in the other regions. In the lower region of the hypocotyl the endodermis becomes primary again and finally disappears. In somewhat older seedlings, according to Rüggeberg (10), the primary endodermis extends within a few millimeters of the apex of the hypocotyl and assumes the secondary state as soon as the cortex no longer offers protection to the vascular tissue.

The central cylinder of root and lower hypocotyl is made up of a diarch protoxylem plate with alternating phloem groups, a single-layered pericycle, and a band of parenchyma between xylem and phloem (fig. 13). In the upper hypocotyl, however, phloem and xylem form collateral bundles while the center of the stele is occupied by a pith (fig. 14, B; fig. 18, B). The change in the arrangement of the vascular tissue which takes place in the upper hypocotyl is described in detail later.

The pericycle of the young stele forms a single-layered concentric ring next to the endodermis (fig. 13). Its cells are uniform in shape and more or less rectangular. Its embryonic progenitors are like the other parenchyma cells. Soon, however, they begin to divide and elongate axially. They remain small in cross section while the cells of the endodermis greatly enlarge.

Differentiation of the primary vascular tissue takes place close behind the growing region. Here the procambium forms a dense

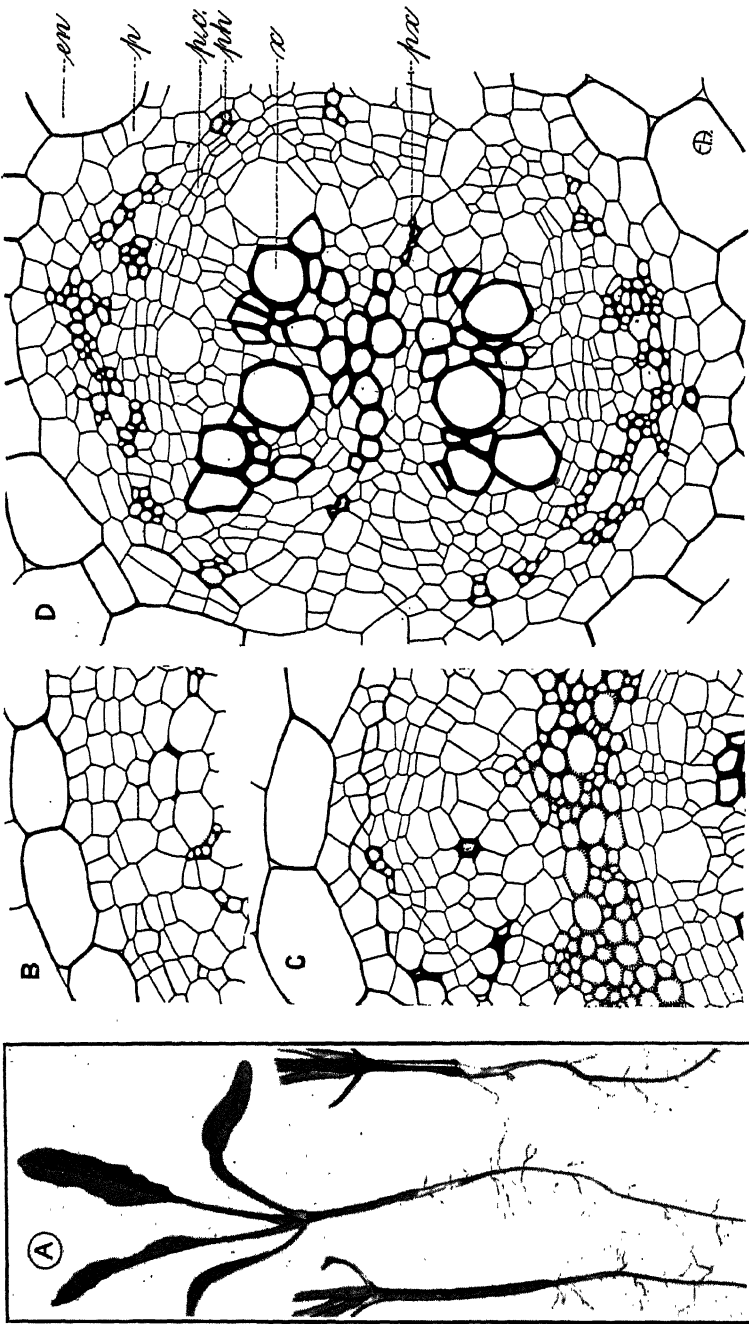


FIG. 12.—4. Beet seedlings about 15 days old. Seedling at the right shows the beginning of the sloughing off of the cortex. *B*. Development of a supernumerary cambium from cells of the pericycle. $\times 305$. *C*. Development of a supernumerary cambium from the cells of the phloem parenchyma. $\times 305$. *D*. Cross section through vascular tissue of young seedling. No supernumerary cambium has yet been formed. *en*, endodermis; *p*, pericycle; *p. c.*, primary cambium; *ph.*, phloem; *x*, xylem; *p. x*, protoxylem. $\times 305$

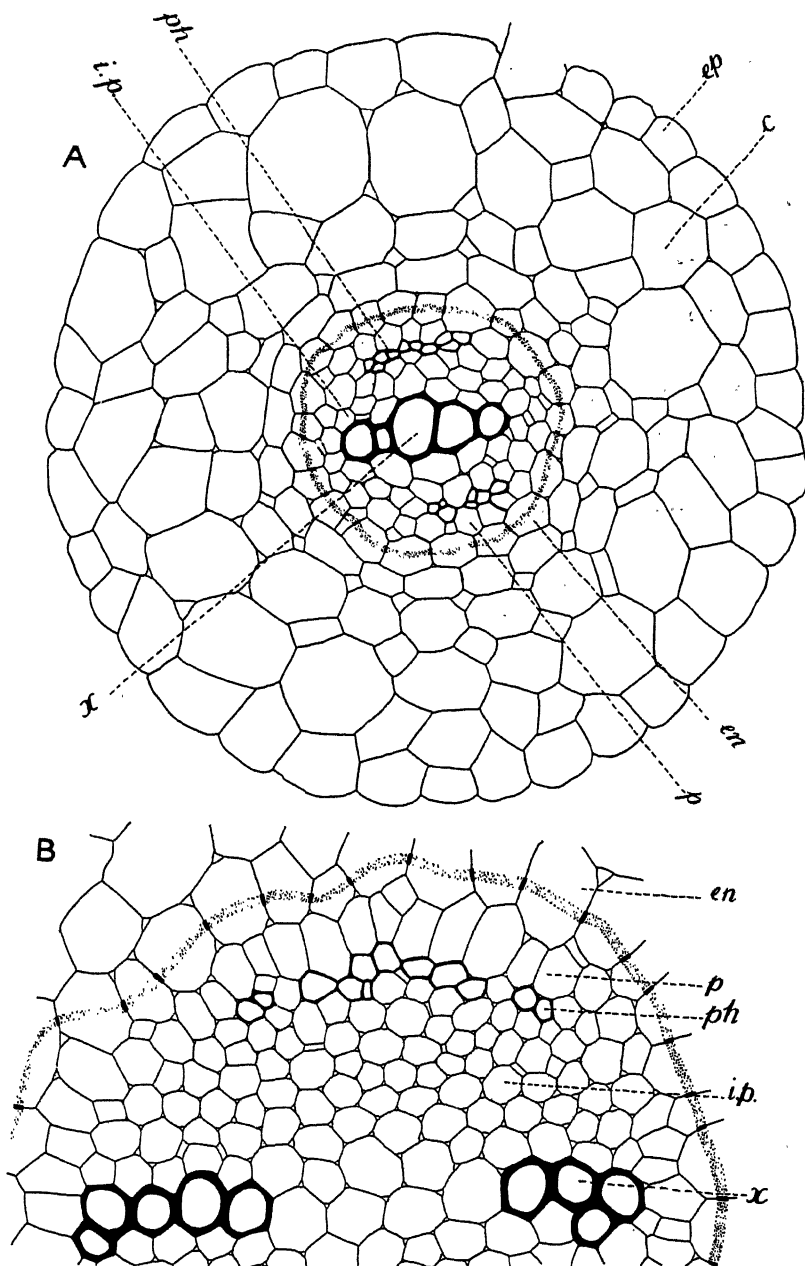


FIG. 13.—A. Cross section through young beet seedling. $\times 355$. c, cortex; ep, epidermis; en, endodermis; ph, phloem; i. p., interstitial parenchyma; p, pericycle; x, xylem. B. Partial cross section of young beet seedling. $\times 476$. The interstitial parenchyma in which the primary cambium originates is much more developed here than in A

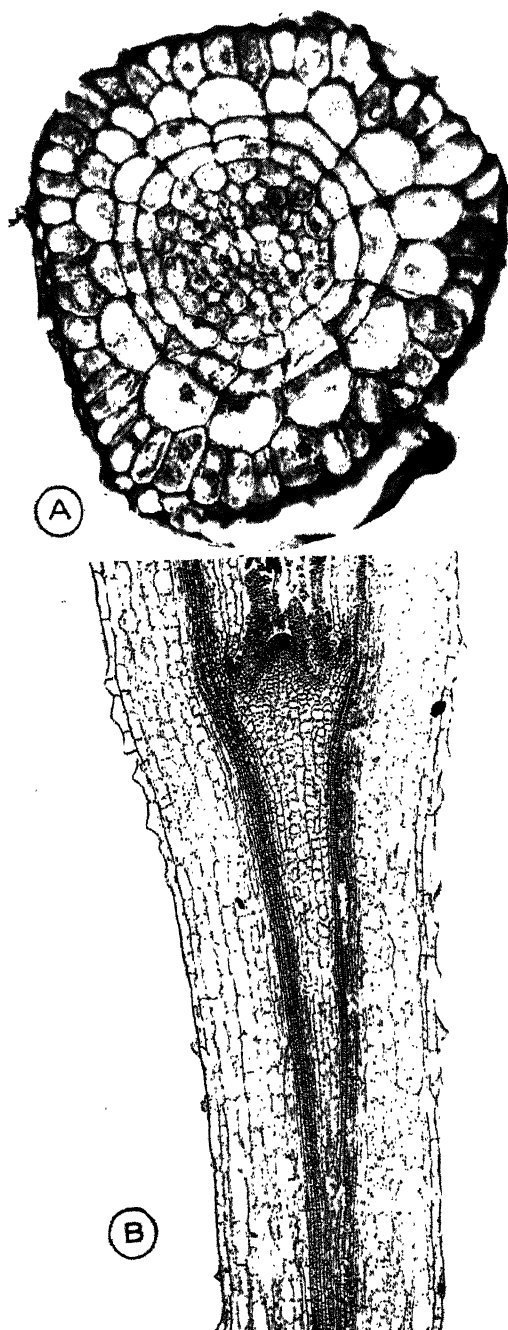


FIG. 14.—A. Cross section of young beet root. $\times 465$. Note the formation of the first two sieve tubes of the vascular tissue. (See legend of Figure 15, A, for identification of issues.) B. Median longitudinal section of young seedling. $\times 46$

tissue composed of elongated thin-walled cells. Specialization in these cells begins at a distance of about 2 millimeters from the calyptragen. Two cells, lying adjacent to the pericycle and separated from one another by an angle of 180 degrees, have enlarged and divided to form the first sieve tubes and companion cells of the primary phloem (fig. 14, A; fig. 15). The sieve tube commonly abuts on the pericycle, but in the upper hypocotyl, according to Seeliger (13), the companion cell lies adjacent to the pericycle and the sieve tube next to it. Soon after the first phloem cells have differentiated, two other procambium cells which lie to the right and left of the sieve tubes undergo changes and mature into the first elements of the protoxylem. Differentiation in the protoxylem progresses centripetally until the two protoxylem points meet in the center to form the primary xylem plates. From now on xylem cells mature to the right and left of the xylem plate until all the cells of the primary wood have been formed (fig. 16). The first-formed xylem cells are narrow elongated elements with sloping or transverse end walls. They have secondary wall thickenings in the nature of rings or spirals. The later-formed cells are larger

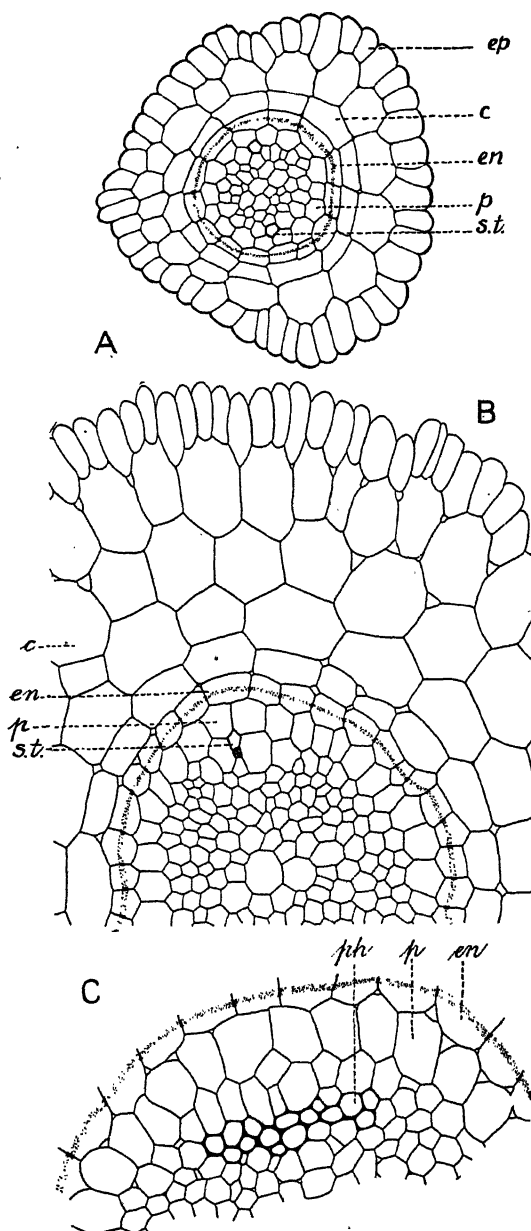


FIG. 15.—A. Cross section through young seedling root of beet. $\times 335$. *ep*, epidermis; *c*, cortex; *en*, endodermis; *p*, pericycle; *s. t.*, sieve tube. Notice that the cortex is very narrow. B. Cross section through large seedling root of the same age. $\times 335$. The first sieve tube and companion cell have differentiated, but no xylem. C. Cross section of a somewhat older root. $\times 580$. Note the increase in the amount of phloem

in cross section but shorter longitudinally. They are reticulate or form transition stages to the spiral or ringed forms. The first-formed xylem elements are the protoxylem while the later-formed cells constitute the metaxylem of the primary wood.

Differentiation in the phloem is less readily followed, because of the small size of the elements. Seeliger (13) states that the metaphloem differentiates from procambium one or more cells to the inside of the pericycle. This later-formed phloem is made up of sieve tubes, companion cells and phloem parenchyma. In the region of the hypocotyl, xylem and phloem form collateral bundles in which the protoxylem is endarch. The change from the exarch condition in the root to the endarch condition in the upper hypocotyl is very abrupt, with the transition region extending over only a few millimeters. In this process, progressively differently situated procambium cells mature into vascular tissue. The two poles of the xylem plate, which in the root meet in the center, become separated, because of the failure of the procambium in this region to

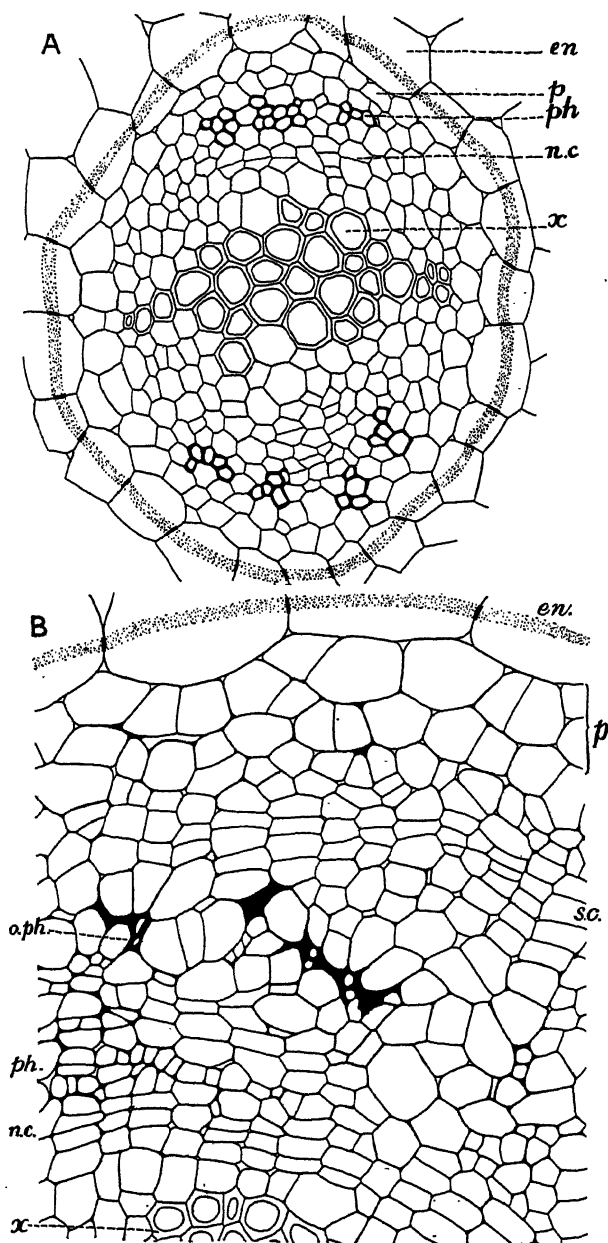


FIG. 16.—A. Cross section through young beet seedling root, showing development of primary xylem plate and the initiation of the primary cambium. *en*, endodermis; *p*, pericycle; *ph*, phloem; *n.c.*, normal primary cambium; *x*, xylem. $\times 373$. B. Cross section through older seedling, showing development of the secondary cambium. *en*, endodermis; *p*, pericycle; *s.c.*, secondary cambium; *o.ph.*, obliterated protophloem; *ph*, phloem; *n.c.*, normal primary cambium; *x*, xylem. $\times 373$

mature into xylem elements. Xylem differentiation becomes instead more prominent on either side of the two strands whereby the latter change their shape at first to triangular (fig. 17) and then to oval. Later-formed elements appear more and more to the inside until in the upper region of the hypocotyl the change from the exarch to the endarch arrangement has been completed. The phloem is also affected in this change from root to stem structure. The two phloem groups become divided, each forming two strands. Then two and two halves of opposite groups approach each other and come to lie external to the xylem with which they now form collateral bundles. While the central cylinder increases in size the cells of the cortex

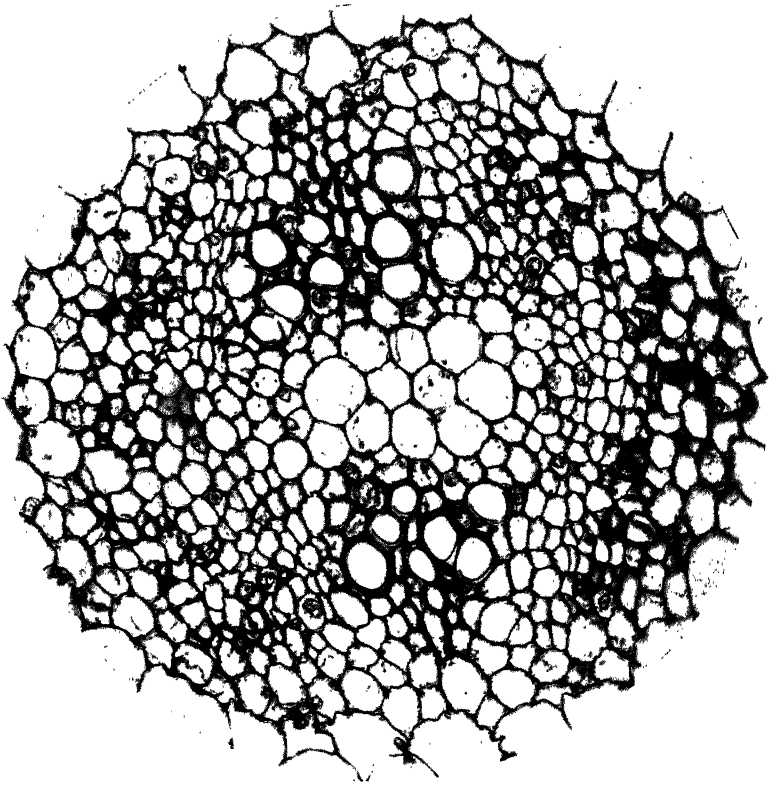


FIG. 17.—Cross section through hypocotyl of beet seedling. The xylem is changing from the exarch to the endarch condition. $\times 435$

grow but little. They are at first passively stretched but later rupture and collapse. These changes are externally visible by the appearance of fine fissures which gradually widen, and finally the cortex is sloughed off.

This process takes place earlier in the root region than in the hypocotyl. In the latter the changes in the cortex due to radial expansion of the stele cause first an extension of the endodermis from the lower hypocotyl to the immediate vicinity of the growing region of the crown. Concomitant with the sloughing off of the cortex is the development of a periderm, which takes over the protective function of the primary cortex. Since the protoxylem is

formed in a region where there is much change and enlargement in radial and tangential directions, the elongation of the surrounding tissue causes the rings of the elements to be pulled farther apart, and, as the stretching continues, the cells may be flattened until the lumen is closed. Metaxylem and wood-parenchyma remain unchanged, but, according to Seeliger (13), suffer displacement due to radial contraction of the root, which takes place simultaneously with the development of the secondary tissues.

SECONDARY GROWTH

The primary growth of the beet is concluded with the appearance of the second pair of leaflets; this normally takes from 10 to 12 days after the seed is planted. The largest diameter of the beet at that stage is at most only a few millimeters, while that of the mature beet is 15 centimeters or more. This increase in thickness is the result of cambial growth accompanied by independent cell division and cell enlargement of the parenchyma.

Cambial activity embraces two distinct phases: Differentiation and growth of the primary cambium and development of secondary cambiums. The primary cambium forms the innermost annular zone in the beet, while the secondary cambiums form a large number of supernumerary rings, of which, however, only the inner five or six mature their tissues. Since the origin of the primary and secondary cambiums differ, their development will be studied separately.

DEVELOPMENT OF THE PRIMARY CAMBIUM

In the following description, unless stated differently, the anatomical picture is that of the lower hypocotyl or upper root region. Since growth and maturation of the tissues takes place acropetally, sections lower down will give younger developmental stages, those higher up more advanced ones.

When a seedling is about 10 days old and the second pair of leaflets becomes visible (fig. 12, A), the parenchyma cells between the primary xylem and phloem begin to elongate axially and undergo tangential division. A new meristematic zone thus arises which becomes the primary cambium and as such develops xylem and phloem in the normal manner. This cambium appears at first in the region of the two phloem poles, but gradually extends laterally over the two protoxylem points. In the latter case, however, the divisions which give rise to the cambium take place in the pericycle.

The xylem formed by the cambium unites intimately with the metaxylem of the primary wood (fig. 18, A). Occasionally, according to de Bary (1), a layer of parenchyma is interpolated between primary and secondary xylem. The zone in front of the protoxylem points remains free from secondary xylem; here the cambium forms parenchyma tissue which forms the two primary medullary rays.

Simultaneously with the formation of secondary xylem, the cambium is forming phloem centrifugally. This secondary phloem, like the xylem, becomes continuous with the primary tissue and indistinguishable from it (fig. 12, D). While the secondary phloem is forming, the primary phloem parenchyma enlarges and divides, thereby forcing the groups of sieve tubes apart; and since the latter have ceased development, they are at first stretched and finally obliterated

(fig. 12, C). The primary cambium continues active growth, but its office is soon to be yielded to another meristematic layer—the secondary or supernumerary cambium.

DEVELOPMENT OF THE SECONDARY CAMBIUM

The origin of the secondary cambium is not uniform for different regions of the beet, and even similar regions show pronounced deviations. The situation is perhaps best portrayed by the following remark of Seeliger (13) in the summary of his detailed ontogenetic studies: "In the development of supernumerary tissues it is not the morphological origin of a cell but its topographic relation to the axis and the neighboring tissues which determines its future."

In the early development of the seedling the first phloem cells develop adjacent to the pericycle, and the later developed metaphloem is separated from the pericycle by a single layer of undifferentiated procambial tissue. The cells of this layer enlarge and subsequently divide, thereby interpolating an ever-widening band of parenchyma between pericycle and phloem (fig. 15, B). This band of

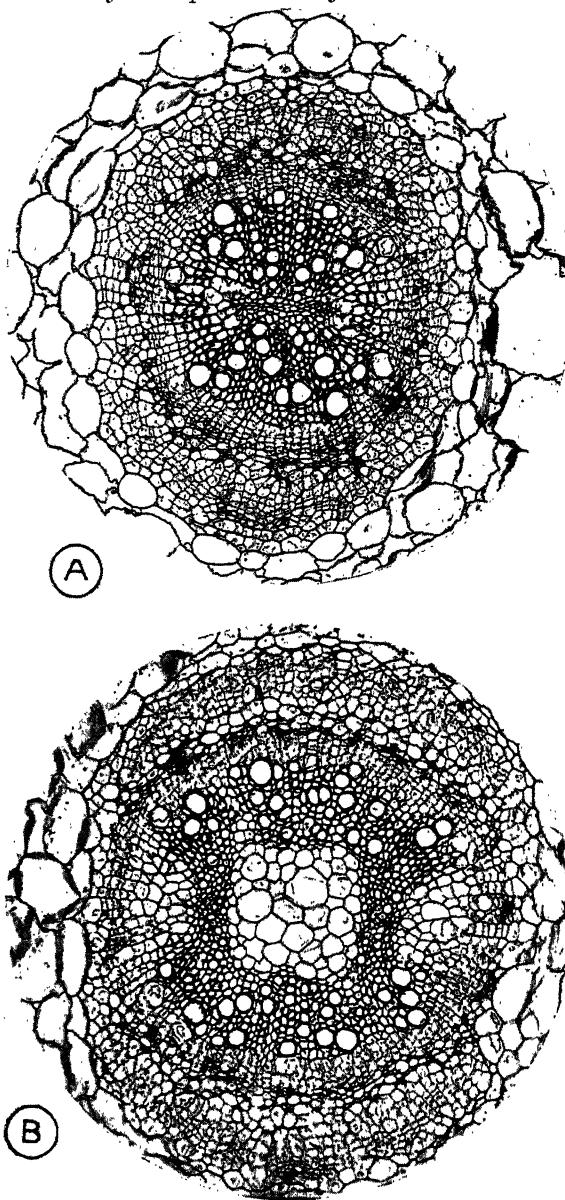


FIG. 18.—A. Cross section through a young beet seedling, showing development and activity of the primary and secondary cambiums. $\times 85$. (See legend of Figure 16, B, for identification of tissues.) B. Cross section through the upper hypocotyl of the same seedling. $\times 85$

parenchyma is not of uniform width, partly because cell division in the primary phloem parenchyma causes certain of the phloem groups to remain close to the pericycle. Seeliger does not mention this mode of development, but derives all this tissue from divisions in the phloem parenchyma. Soon there appear periclinal division walls in certain cells of this band, initiating the development of a secondary cambium. In the region of the protoxylem points, of course, the secondary cambium originates just as does the primary one in the pericycle (fig. 12, B).

This mode of development is characteristic for the root and the lower hypocotyl. In the upper hypocotyl the cambium arises altogether in the pericycle, while the central hypocotyl shows transition stages with more and more pericycle cells taking part in cambium formation as the apical region is approached.

Once initiated the cambium will produce an annular zone of bundles and parenchyma tissue. Since, however, additional cambiums are formed, the behavior of the first cambium initial differs from the normal behavior of cambium cells. When the cambium initial undergoes the first division, the outer of the two daughter cells becomes the initial of a new supernumerary cambium, while the inner daughter cell divides further and produces xylem, phloem, and medullary ray tissue. This process is repeated until all supernumerary cambiums have been formed. However there is no uniform method governing the formation of the supernumerary cambiums. Often sections of two supernumerary cambiums originate simultaneously, one from an inner, the other from a more peripheral phloem parenchyma cell. Since most of the supernumerary cambiums of the beet are initiated in quick succession, a beet no thicker than a pencil contains practically all annular zones of growth developing simultaneously.

PERIDERM DEVELOPMENT

The periderm of the beet always develops from cells of the pericycle. Its formation is initiated when the seedling has about five pairs of leaves, that is, at a time when the supernumerary cambiums are forming and the primary cortex is being sloughed off, and since this process takes place first in the root zone, the periderm develops acropetally, extending gradually into the hypocotyl. Periderm development begins with a conversion of the cells of the pericycle, by tangential divisions, into a band of meristematic tissue, which constitutes the phellogen or cork cambium. From the phellogen are formed, by reciprocal division, cork cells outside and phelloderm cells inside. The number of phelloderm cells, however, is smaller than the number of cork cells, since the latter are constantly being sloughed off and must be replaced. On the whole the periderm forms a thin covering from five to eight cells wide (fig. 19). The individual cork cells have the form of a parallelepiped with a five or six sided base. The height is less than the diameter of the bases, thus giving the cells a flattened appearance. The walls of the periderm cells are thin and suberized, except the middle lamella which is lignified.

DEVELOPMENT AND GROWTH OF THE ANNUAL RINGS

As previously stated, the peculiar zonation noticed in a cross section of a beet is produced by concentric rings of vascular tissue inclosing broad bands of parenchyma. Near the periphery, however,

the rings are very narrow and the tissues just in the process of differentiation (fig. 20). By examining the different rings in centripetal order, one can easily follow out the ontogeny of a ring.

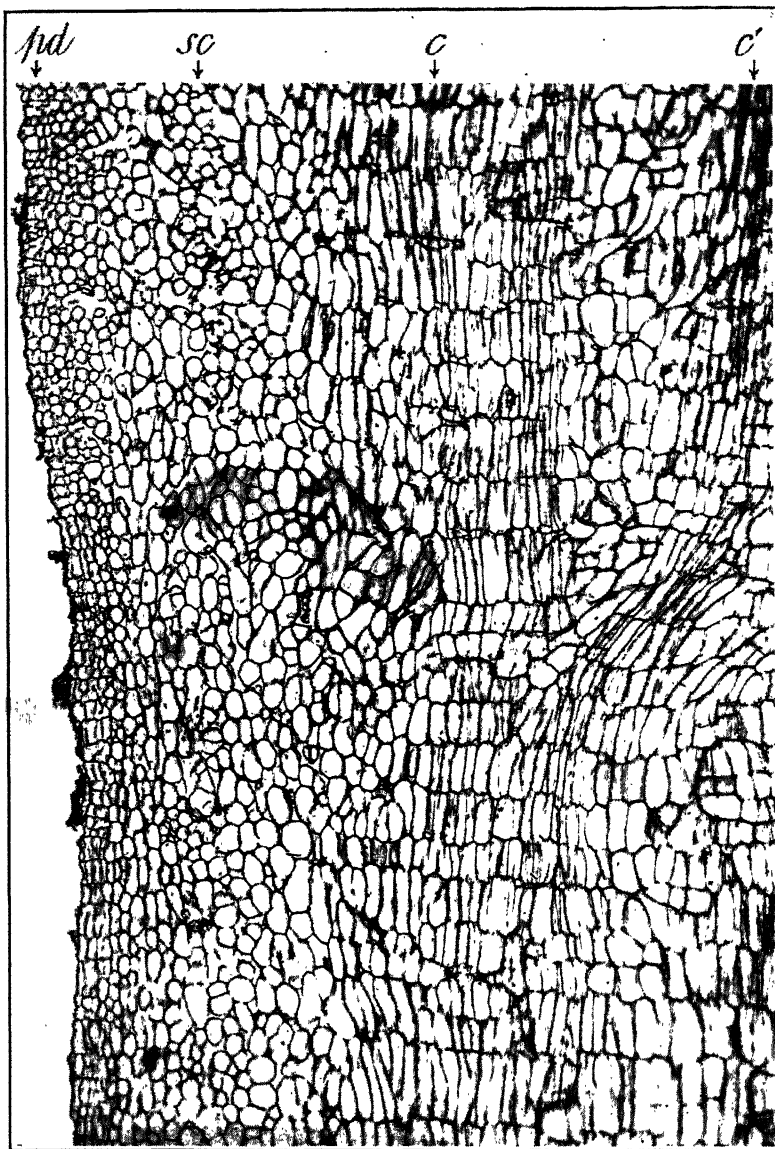


FIG. 19.—Radial section through periphery of mature beet, showing periderm, cortex, and cambium of the first ring. *pd*, periderm; *s. c.*, secondary cortex; *c*, cambium; *c'*, cambium of second ring. $\times 270$

The development and growth of the individual rings follows, in principle, the differentiation processes of ordinary collateral bundles, but these are modified because of the interpolation of large amounts of storage parenchyma inside the bundles.

The youngest ring, nearest the periphery, is composed of a multi-seriate cambium in which here and there a few cells have matured into small groups of sieve tubes and companion cells (fig. 21). In the second ring phloem differentiation becomes quite general, and

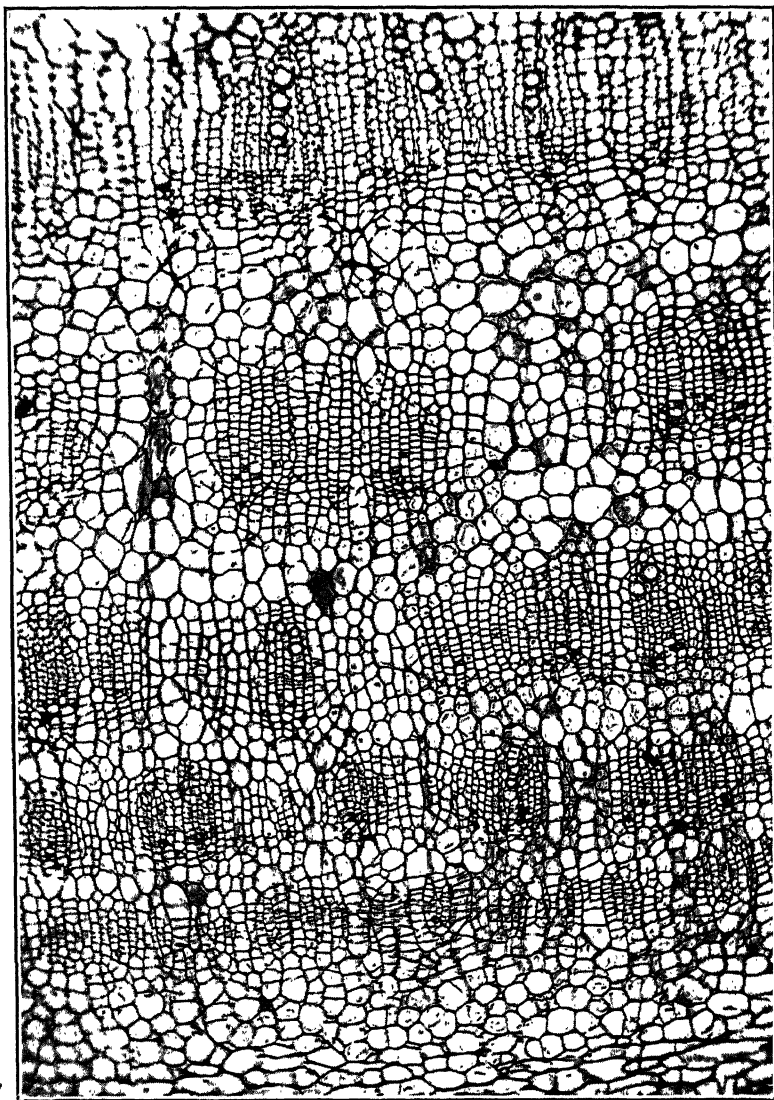


FIG. 20.—Cross section of peripheral zone of mature beet. The rings are composed mostly of undifferentiated cambium and some phloem. $\times 90$

even in subsequent ones the extensive development of phloem dominates the anatomical picture. Following the differentiation of the first sieve tubes, phloem parenchyma is formed, and this subsequently divides and enlarges, pushing the first-formed groups of sieve tubes farther away from the vascular ring and finally obliterating them.

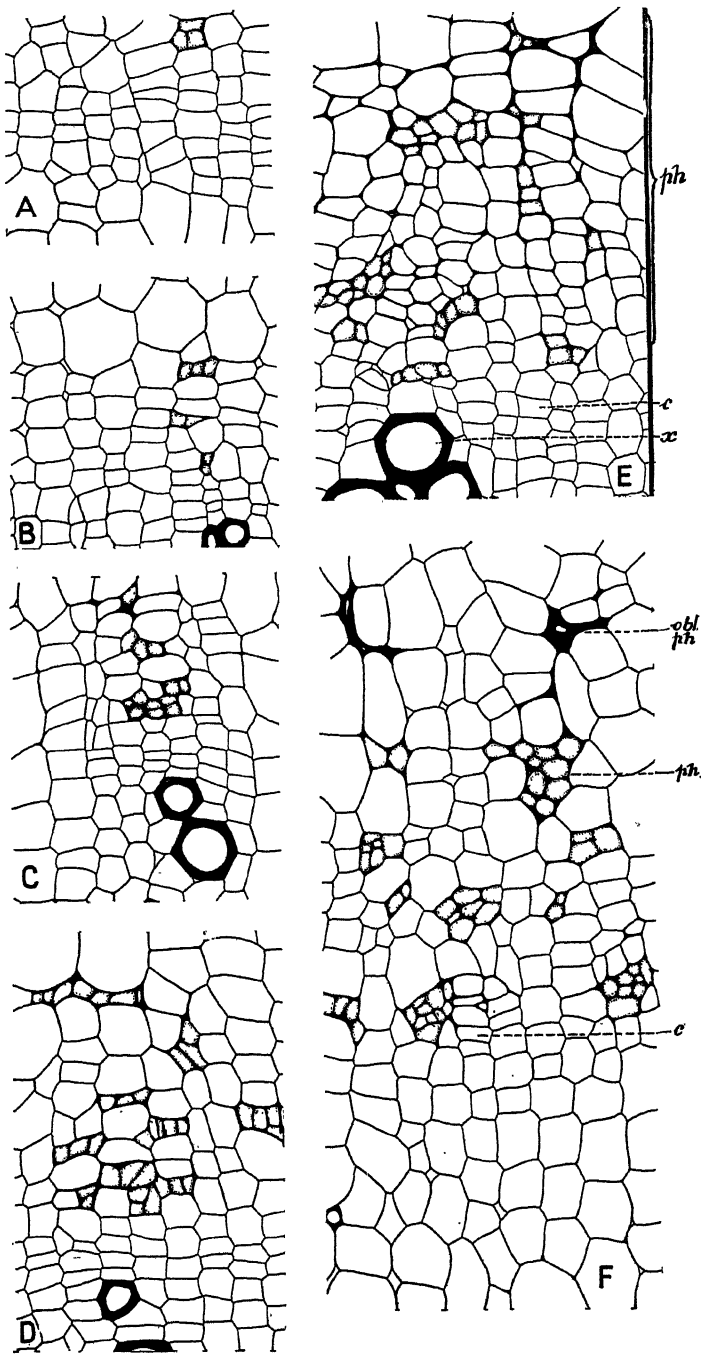


FIG. 21.—Ontogeny of the phloem of sugar beet. $\times 317$. *A*, first peripheral ring; *B*, second ring; *C*, third ring; *D*, fourth ring; *E*, fifth ring; *F*, fifth ring, but from a different part of the beet. The phloem shows a very marked degree of development compared to the xylem. The outermost phloem groups in *F* are already obliterated. $\times 320$

In general, the groups of sieve tubes appear in narrow radial bands broken up radially and tangentially by the larger cells of the phloem parenchyma.

Centripetally the cambium develops a broad band of parenchyma in which appear the first xylem cells; this occurs usually only after considerable phloem has been formed. In certain bundles, phloem and xylem differentiation appears to be reciprocal, but most often the appearance of xylem is belated. After the first few xylem cells have been formed, the cambium matures additional parenchyma and occasionally a xylem cell. Cell division in the parenchyma between the xylem continues irregularly, and as a result the xylem cells became displaced radially and tangentially and project far into the parenchyma (fig. 22). The cambium between the bundles gives rise to large-celled medullary-ray tissue. Where bundles are very close together the ray cells are small and radially elongated.

The rings of vascular tissue are separated by broad bands of storage parenchyma. In the peripheral zone these bands are not more than one or two cells wide, and in places the phloem of the next inner ring abuts on the cambium of the outer ring (fig. 23). Since each supernumerary cambium is the direct descendant of the next older cambium, and since the first-differentiated phloem groups are sieve tubes, the band of interzonal parenchyma is ultimately the product of centripetal cambial growth.

A close examination of the interzonal parenchyma shows that it is in reality made up of three regions: An outer, comparatively broad zone, containing scattered xylem cells; an intermediate, purely parenchymatous zone; and an inner zone containing obliterated phloem (fig. 2, A). As xylem differentiation is strictly centripetal the innermost xylem cells constitute the inner limit of the first zone, which is thus closely related to the vascular ring. The broad intermediate zone has been formed by cell division and cell enlargement of the parenchyma cells differentiated by the cambium previous to xylem formation. Finally, the inner zone is delimited centrifugally by the obliterated phloem and consists chiefly of phloem parenchyma. It is therefore the product of centrifugal growth of the older ring.

SUMMARY

The sugar beet is an elongated more or less pear-shaped body composed morphologically of crown, neck, and root. In cross section it appears to be made up of a number of annular zones or rings of growth, separated by bands of storage parenchyma. Only the four or five inner rings mature their tissues, while the peripheral ones remain in a more or less meristematic condition.

The center of the beet is occupied by a solid, more or less star-shaped, core of which the innermost part constitutes the primary xylem plate. The latter is either directly continuous with the secondary xylem of the core, or is separated from it by a concentric ring of parenchyma of varying width.

The young seedling beet has a central strand of vascular tissue inclosed by a cortex and bounded at the periphery by an epidermis. The central strand is made up of a diarch protoxylem plate with alternating phloem groups, a single-layered pericycle and a band of interstitial parenchyma between xylem and phloem.

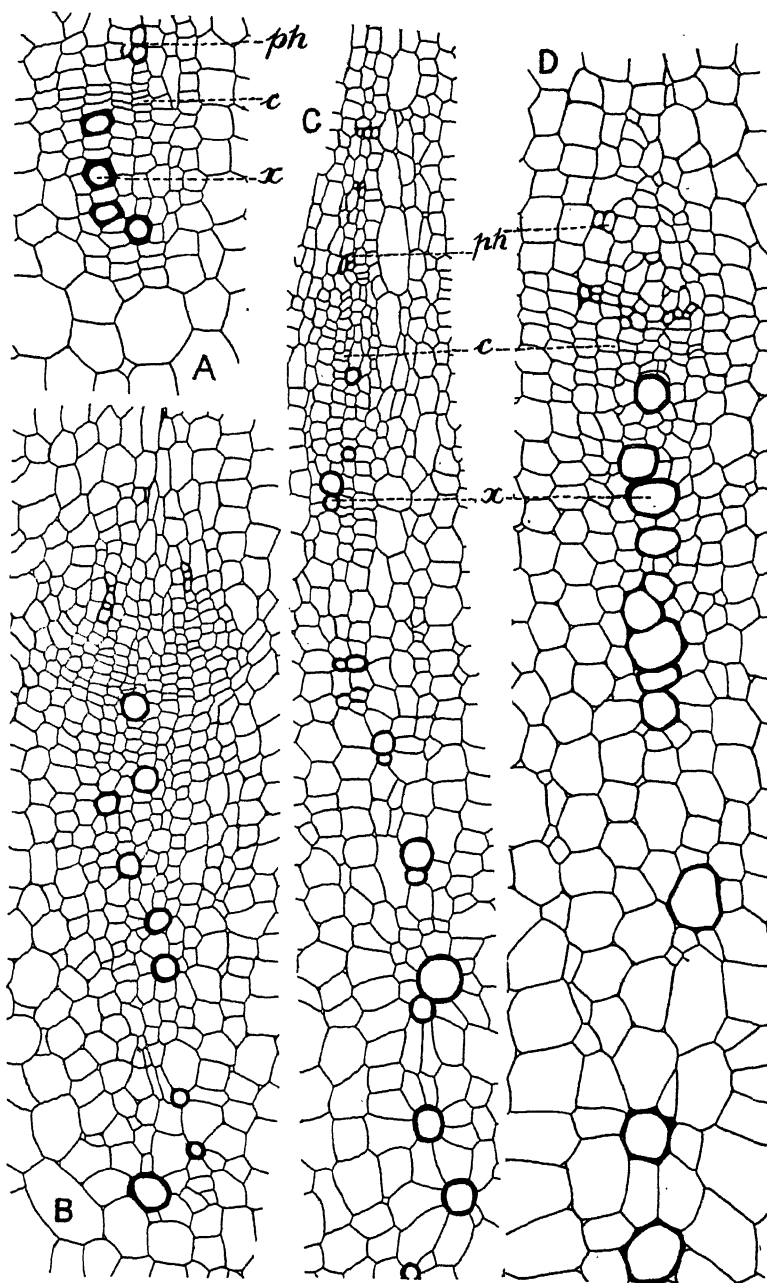


FIG. 22.—A. Young peripheral bundle of mature beet. $\times 510$. *ph*, phloem; *c*, cambium; *x*, xylem. B. Older bundle, with parenchyma developing between the xylem cells. $\times 100$. C. Very narrow bundle of the same age. A large amount of parenchyma has become interpolated between the vessels, forcing them farther and farther apart. $\times 100$. D. Bundle of an older ring, which shows a larger amount of xylem. $\times 180$

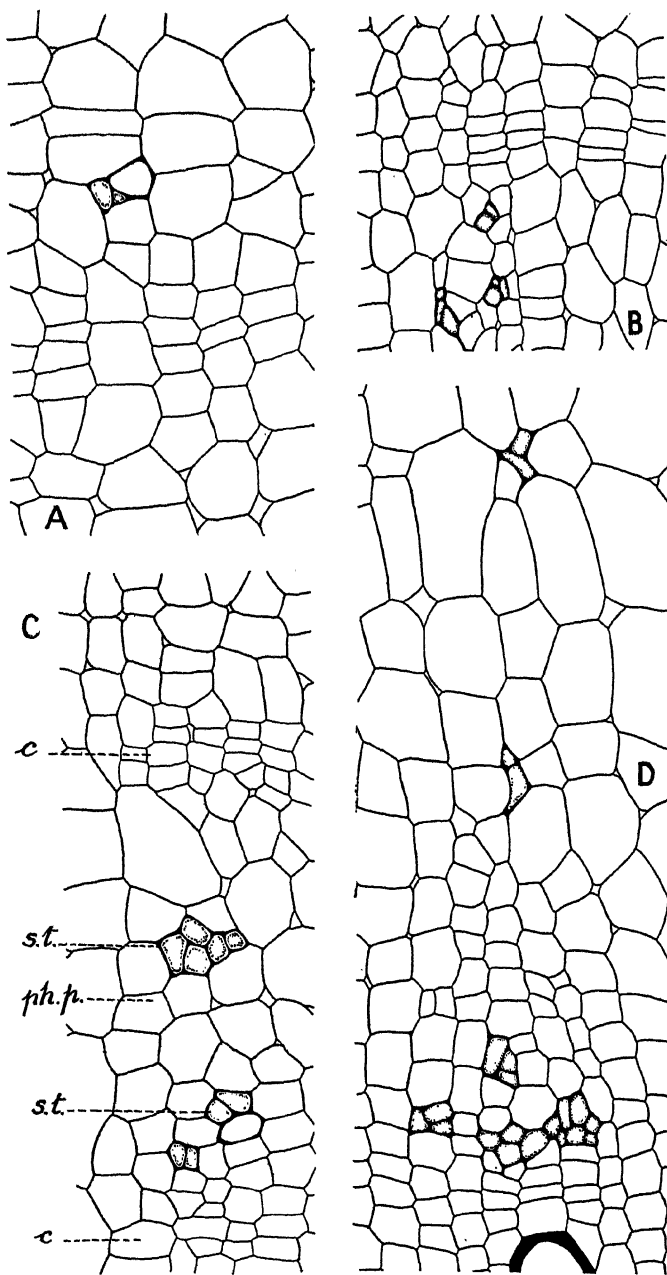


FIG. 23.—Ontogeny of the interzonal parenchyma of beet. *A.* Peripheral zone of an actively growing beet. The outermost ring comprises only one layer of cambium. The cambium of the second ring has already formed a group of sieve tubes and parenchyma. Note that the group of sieve tubes lies next to the cambium of the first ring. *B.* A more advanced stage than in *A.* *C.* The outer cambium has formed parenchyma centripetally. In the next inner ring the first sieve tubes have become separated from later-formed ones by phloem parenchyma cells. *c.* cambium; *st.* sieve tube; *ph. p.* phloem parenchyma. *D.* A more advanced stage. The first-formed sieve tubes project far into the parenchyma of the ring.

Secondary growth of the beet involves the activity of a primary cambium and of secondary cambiums.

The primary cambium gives rise to the innermost annual ring in the beet. It arises in the interstitial parenchyma, except, of course, in the region opposite the two protoxylem points where it is derived from the pericycle.

The first secondary cambium arises in root and lower hypocotyl from cells of the primary phloem parenchyma. Occasionally undifferentiated procambium cells between pericycle and phloem parenchyma contribute to its development. In the upper hypocotyl it is derived from the pericycle, and in the intermediate hypocotyl both pericycle and phloem parenchyma contribute to its development. In the region opposite the protoxylem points, both primary and secondary cambiums are descendants from pericycle tissue.

All other supernumerary cambiums stand for the most part in direct lineage with the first secondary cambium.

The periderm is derived from the pericycle. It forms phellogen and phelloderm cells in reciprocal fashion.

Practically all the supernumerary cambiums for the annular rings of the mature beet have been formed while the latter is no thicker than a lead pencil. The enormous increase in the diameter of the beet is due to cell division and cell enlargement taking place simultaneously in all the rings.

The degree of development attained by the vascular tissue of a ring and the separating band of interzonal parenchyma varies greatly with different beets. In any given one, however, the innermost rings have the broadest band of parenchyma.

The interzonal parenchyma in its entirety is made up of three regions: An outer zone containing scattered xylem cells, a central purely parenchyma zone, and an inner zone containing obliterated phloem. The first two zones have been formed by centripetal growth of the outer ring, while the third zone is the product of centrifugal growth of the older inner ring.

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INSECTICIDAL VALUE OF CERTAIN WAR CHEMICALS AS TESTED ON THE TENT CATERPILLAR¹

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INTRODUCTION

At the request of the Chemical Warfare Service of the War Department, the writer was detailed by the Bureau of Entomology of the Department of Agriculture to make a study of the chemical compounds developed by the Chemical Warfare Service during and since the World War, to determine their value as insecticides. The War Department has developed many new and interesting compounds, but the majority of them seem to have no practical value as insecticides and hence have not been studied in detail. All of the available compounds that were thought to have any possible value as stomach poisons, contact insecticides, or fumigants were tested for toxicity to insects and host plants. This paper is a general report of the experiments conducted with the various materials to learn something of their usefulness as stomach poisons and contact insecticides. The report on fumigation will be presented later.

Owing to the fact that a rather large number of compounds were to be tested, it was decided to conduct the experiments in such a way that only a general idea of the toxicity of the compounds to insects and plants might be obtained, no attempt being made to determine the exact toxicity of any compound. It was considered that the results obtained with 10 to 25 insects in each test would be sufficient to furnish a basis for further study.

The work reported here was conducted in cooperation with the Chemical Warfare Service at Edgewood Arsenal, Md., in 1924.

EXPERIMENTS ON STOMACH POISONING

Eastern tent caterpillars (*Malacosoma americana* Fab.) were very abundant at the Arsenal, and as they are considered to be somewhat resistant to arsenicals, they were used in this study, and proved to be excellent material.

The chemical compound to be tested as a stomach poison was sprayed or dusted on the leaves of small twigs of wild cherry, the favorite food plant of this insect. The sprayed branches were placed in vials of water. The vials were then inclosed in cylindrical wire cages 4 inches in diameter and 10 inches in height. Twenty-five third-grown to half-grown caterpillars were put into each cage with the sprayed foliage. Notes were taken daily, the relative amount of foliage consumed and the time of death being carefully noted in each case.

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CHEMICAL COMPOUNDS USED

The compounds tested were largely organic arsenicals, many of which were the oxides of gases and smokes developed during the World War. The cost of some of these compounds would prohibit their use as insecticides, but in this study the cost was not taken into consideration.

Following is a list of the compounds used, with a brief description of some of their chemical and physical properties:

1. DIPHENYLAMINO ARSENIUS OXIDE, $[\text{NH}(\text{C}_6\text{H}_5)_2\text{As}]_2\text{O}$. A light-yellow amorphous compound, finely divided into extremely small particles. It mixes well with water, if a paste is first made with a small amount of water and more water is then added slowly to this paste until the desired dilution is reached. This chemical spreads evenly over the foliage, and remains well in suspension. Great caution must be used in handling it, for inhalation of it irritates the nasal passages and throat and causes sneezing.

2. DIPHENYL ARSENIUS OXIDE, $(\text{C}_6\text{H}_5)_2\text{As}_2\text{O}_3$. A white powder of very fine crystals which settle out very rapidly from an aqueous suspension and collect in small patches on the sprayed foliage.

3. CHLOROVINYL ARSENIUS OXIDE, $\text{ClHC}=\text{CHAsO}$. A white flaky crystalline compound. Does not mix well with water; it settles very rapidly in water, and the spray deposit on foliage is uneven and patchy. If the crystals remain on the skin they cause a slight irritation, and in some cases blistering. The fumes are very irritating to the nose and throat.

4. CALCIUM ETHYL ARSONATE (calcium salt of ethyl arsonic acid), $\text{CaC}_2\text{H}_5\text{AsO}_3$. A very fine powder which mixes readily with water and remains well in suspension. The spray deposit is spread evenly over the leaf.

5. ETHYLENE DITHIO-CYANATE, $\text{NCSCH}_2\text{CH}_2\text{SCN}$. A heavy crystalline material, which settles very quickly after being mixed with water. It does not form an even deposit over the leaf.

6. 2-4-6 TRICHLOROPHENYL BENZYLCHLORAMINE, $\text{Cl}_3\text{C}_6\text{H}_2\text{NClCH}_2\text{C}_6\text{H}_5$. A light-brown gummy compound, which forms small pellets when mixed with water.

7. 2-4-6 TRICHLOROPHENYL ACETYL CHLORAMINE, $\text{Cl}_3\text{C}_6\text{H}_2\text{NClCOCH}_3$. Is similar to trichlorophenyl benzylchloramine.

8. CALCIUM METHYL ARSONATE (calcium salt of methyl arsonic acid), $\text{CaCH}_3\text{AsO}_3$. Similar to calcium ethyl arsonate.

9. PHENYL ARSENIUS OXIDE, $\text{C}_6\text{H}_5\text{AsO}$. Similar to diphenyl arsonic oxide.

10. DIMETHYL ANILINO ARSENIUS OXIDE, $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{AsO}$.

11. p-DIMETHYL AMINO BENZALDEHYDE, $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CHO}$.

12. COPPER STEARATE, COPPER RESINATE, MERCURY STEARATE, LEAD STEARATE, LEAD RESINATE, MERCURY RESINATE, and ZINC RESINATE, were very fine powders which had been passed through a 160-mesh sieve. They were exceedingly hard to mix with water. Mixture with water was accomplished only by first making a paste and then adding slowly the required amount of water. These materials spread very well over the entire leaf surface, forming a thin even film.

EFFECT ON TENT CATERPILLAR AND THE BEAN PLANT

The effect of the compound on the plant could not be determined where wild cherry was used, because the branches were detached from the plant. Beans were used because they could easily be grown in pots, could be handled without difficulty, and are very susceptible to injury by arsenical compounds.

Table 1 gives a list of the compounds used and their comparative toxicity to tent caterpillars and host plants. In Table 1 "concentration" is the amount in grams of the material in 100 c. c. of distilled water, except where dusts were used. Where dusts were used the material was mixed with kaolin and applied in the proportion given, as, for example, one part of material to five of kaolin. The time in days refers to the time between the first feeding and the death of the last insect, or to the time when the experiment was closed.

Control experiments were conducted with unsprayed foliage so that the normal death rate might be observed. Lead arsenate was used as the standard for comparison with the materials mentioned above.

TABLE 1.—Comparative toxicity of a number of organic compounds to tent caterpillars and bean plants when the compounds were applied as sprays to kill by stomach poisoning

Material used	Concentration (gms. per 100 c. c. of water)	Number of insects in test	Number dead	Days from first feeding to last death	Effect on plant	Foliage eaten
Diphenylamino arsenious oxide.	0.5	25	25	5	None.....	Small amount.
Do.....	1.0	25	25	4	do.....	Do.
Do.....	2.0	25	25	7	do.....	Do.
Do.....	(^a)	25	25	6	Very slight.....	Do.
Do.....	(^b)	25	25	6	None.....	Do.
Diphenyl arsenious oxide.	.5	25	25	4	Severely burned.....	Do.
Do.....	1.0	25	25	4	do.....	Do.
Do.....	2.0	25	25	4	do.....	Do.
Do.....	.1	25	25	6	do.....	Do.
Do.....	.25	25	25	3	do.....	Do.
Do.....	(^c)	25	25	3	do.....	Do.
Do.....	(^b)	25	25	5	do.....	Do.
Chlorovinyl arsenious oxide.	.1	25	25	7	do.....	Do.
Do.....	.25	25	23	5	Completely burned.....	Experiment closed
Do.....	.5	25	15	4	do.....	
Do.....	1.0	25	20	4	do.....	
Do.....	2.0	25	17	4	do.....	
Control.....		25	3	9	Food replaced several times.	
Copper stearate.....	.1	25	20	19	None.....	All.
Do.....	.25	25	12	16	do.....	Do.
Do.....	.5	25	9	16	do.....	Do.
Do.....	1.0	25	19	24	do.....	Do.
Copper resinate.....	.1	25	9	9	do.....	Do.
Do.....	.25	25	9	9	do.....	Do.
Do.....	.5	25	8	10	do.....	Do.
Do.....	1.0	25	22	38	do.....	Do.
Mercury stearate.....	.1	25	11	15	do.....	Do.
Do.....	.25	25	14	9	do.....	Do.
Do.....	.5	25	24	13	do.....	Do.
Do.....	1.0	25	15	9	do.....	Very little.
						Experiment closed
						(leaves fell from plant, but not because of burning).
Lead arsenate.....	.1	25	25	13	do.....	One-half.
Do.....	.25	25	25	11	do.....	One-third.
Do.....	.5	25	25	11	do.....	Do.
Do.....	1.0	25	25	16	do.....	Do.
Control.....		25	21	14	do.....	Foliage replaced.

^a Dust (pure).

^b Dust with kaolin (1-10).

^c Dust with kaolin (1-5).

TABLE 1.—Comparative toxicity of a number of organic compounds to tent caterpillars and bean plants when the compounds were applied as sprays to kill by stomach poisoning—Continued

Material used	Concentration (gms. per 100 c. c. of water)	Number of insects in test	Number dead	Days from first feeding to last death	Effect on plant	Foliage eaten
Lead stearate.....	0.1	25	2	3	None.....	All.
Do.....	.25	25	1	3	do.....	Do.
Do.....	.5	25	0	3	do.....	Do.
Do.....	1.0	25	2	9	do.....	Do.
Lead resinate.....	.1	25	11	8	do.....	Do.
Do.....	.25	25	10	11	do.....	Do.
Do.....	.5	25	11	9	do.....	Do.
Do.....	1.0	25	12	8	do.....	Do.
Chlorovinylarsenious oxide.....	^(b)	25	14	4	Severely burned.....	Experiment closed.
Do.....	^(d)	25	19	4	do.....	Do.
Lead arsenate.....	.5	25	25	5	None.....	Fair amount.
Do.....	2.0	25	25	3	do.....	Do.
Kaolin.....	^(e)	25	1	3	do.....	All.
Control.....		25	0	9	do.....	Foliage replaced.
Phenylarsenious oxide	.1	25	25	5	Severely burned.....	
Do.....	.25	25	15	7	do.....	
Do.....	.5	25	25	4	do.....	
Do.....	1.0	25	25	4	do.....	
Calcium methyl arso- nate.....	.1	25	0	3	None.....	All.
Do.....	.25	25	25	5	Severely burned.....	Little.
Do.....	.5	25	15	3	do.....	Experiment closed.
Do.....	1.0	25	25	7	do.....	
Lead arsenate.....	.1	25	25	5	None.....	Fair amount.
Do.....	.25	25	25	4	do.....	
Do.....	.5	25	25	6	do.....	
Do.....	1.0	25	25	8	do.....	
Calcium ethyl arso- nate.....	.1	25	20	13	Severely burned.....	Considerable.
Do.....	.25	25	18	6	do.....	
Do.....	.5	25	25	8	do.....	
Do.....	1.0	25	25	8	do.....	
Ethylene dithio-cya- nate.....	.1	25	9	7	None.....	Considerable.
Do.....	.25	25	0	2	do.....	All.
Do.....	.5	25	0	1	do.....	Do.
Do.....	1.0	25	0	2	do.....	Do.
2-4-6 Trichlorophenyl benzylchloramine.....	1.0	25	24	15	do.....	Do.
Do.....	2.0	25	10	10	do.....	Do.
2-4-6 Trichlorophenyl acetyl chloramine.....	1.0	25	5	3	Tender leaves slightly burned.....	Do.
Do.....	2.0	25	0	5	Slightly burned.....	Do.
p-Dimethyl amino benzaldehyde.....	.1	25	3	8	None.....	Do.
Do.....	.25	25	7	8	do.....	Do.
Do.....	.5	25	3	8	do.....	Do.
Do.....	1.0	25	2	8	do.....	Do.
Dimethyl anilino ar- senious oxide.....	.1	25	15	7	Slightly burned.....	Do.
Do.....	.25	25	25	8	do.....	One-third.
Do.....	.5	25	25	11	Severely burned.....	One-half.
Do.....	1.0	25	25	12	do.....	Do.
Mercury resinate.....	.1	25	0	6	do.....	Do.
Do.....	.25	25	3	8	do.....	Do.
Do.....	.5	25	2	8	do.....	Do.
Do.....	1.0	25	9	8	do.....	Do.
Zinc resinate.....	.1	25	0	5	do.....	Do.
Do.....	.25	25	0	5	do.....	Do.
Do.....	.5	25	1	12	do.....	Do.
Do.....	1.0	25	0	3	do.....	Do.

^a Dust (pure).^b Dust with kaolin (1-10).^d Dust with kaolin (1-25).

Diphenylamino arsenious oxide proved to be the most promising of the materials tried, it being about equal to lead arsenate in toxicity to the insect, and was not injurious to the bean plants even in strong concentrations. When the pure material was dusted on the plant slight injury developed after several days, especially to the tender

leaves and growing tips. Mixed with kaolin in the proportion of 1 part to 10 parts of kaolin, there was no injury to the plants, and a 100 per cent kill resulted in six days.

Diphenyl arsenious oxide chlorovinyl arsenious oxide, and phenyl arsenious oxide, proved to be very toxic to the insect but were also toxic to the plant. Saturated solutions of these materials were made by allowing the compound to remain in water overnight and then filtering it. When this filtrate was used as a spray it burned the plant severely.

Diphenyl arsenious oxide was used with equal parts of hydrated lime. The lime prevented plant injury, but it also greatly reduced the toxicity of the compound to the insect.

When phenyl arsenious oxide was used with lime the injurious effect upon the plant was greatly lessened; severe injury resulted, however, in some cases. The addition of lime reduced the toxicity of the compound to the insect.

Copper stearate and copper resinate, although comparatively nontoxic, seemed to have a decidedly repellent effect upon the insect. It required from 9 to 38 days for the caterpillars to consume the foliage given them, whereas the control insects consumed the same amount of foliage in from 1 to 3 days. It is the opinion of the writer that many of the insects in these tests did not die from poisoning but died from starvation because they would not eat the sprayed foliage. Both copper stearate and copper resinate have excellent spreading and adhering qualities, and it may be practicable to use them as sprays for tender foliage that can not be treated with an arsenical.

EXPERIMENTS ON CONTACT POISONING

Experiments were conducted to determine the toxicity of certain chemical compounds as contact insecticides. The same compounds were used in these tests as were used in the experiments in stomach poisoning. The compounds to be tested were sprayed on half-grown tent caterpillars. The caterpillars were allowed to dry, and they were then placed on unsprayed cherry foliage. Ten larvae were used in each test. Notes were taken daily, and the rate of death observed. Table 2 gives a summary of the results of these experiments.

Diphenylamino arsenious oxide, diphenyl arsenious oxide, and chlorovinyl arsenious oxide proved to be toxic as contact insecticides as well as stomach poisons. However, diphenyl arsenious oxide and chlorovinyl arsenious oxide were extremely toxic to bean plants (Table 1).

Diphenylamino arsenious oxide seems to be a promising contact insecticide as well as stomach poison. Further experiments with this compound are now in progress.

TABLE 2.—Comparative toxicity to tent caterpillars of a number of compounds tested as contact insecticides

Material used	Concentration (grams per 100 c. c. of water)	Number of insects in test	Number dead	Days from treatment to last death
Diphenylamino arsenious oxide.....	0.5	10	10	2
Do.....	1.0	10	10	4
Do.....	2.0	10	10	4
Diphenyl arsenious oxide.....	.1	10	10	3
Do.....	.25	10	10	3
Do.....	.5	10	10	2
Do.....	1.0	10	10	2
Do.....	2.0	10	10	2
Chlorovinyl arsenious oxide.....	.1	10	9	3
Do.....	.25	10	10	1
Do.....	.5	10	10	1
Do.....	1.0	10	10	1
Do.....	2.0	10	10	1
Phenyl arsenious oxide.....	.1	10	0	5
Do.....	.25	10	0	5
Control.....	—	10	0	5
Calcium methyl arsonate.....	.1	10	0	3
Do.....	.25	10	1	3
Do.....	1.0	10	1	3
Calcium ethyl arsonate.....	.1	10	1	5
Do.....	.25	10	1	5
Do.....	1.0	10	0	3
Do.....	.1	10	1	5
Do.....	.25	10	1	5
Do.....	.5	10	10	5
Do.....	1.0	10	6	5
Ethylene dithio-cyanate.....	.1	10	1	5
Do.....	.25	10	1	5
Do.....	.5	10	1	5
2-4-6 Trichlorophenyl benzylchloramine.....	1.0	10	0	3
Do.....	2.0	10	0	3
2-4-6 Trichlorophenyl acetyl chloramine.....	.1	10	1	3
Do.....	.25	10	0	3
Do.....	.5	10	0	3
Do.....	1.0	10	0	10
p-Dimethyl amino benzaldehyde.....	.1	10	0	10
Do.....	.25	10	0	10
Do.....	1.0	10	0	10
Dimethyl anilino arsenious oxide.....	.1	10	2	8
Do.....	.25	10	5	8
Do.....	.5	10	5	8
Do.....	1.0	10	9	6
Copper stearate.....	.1	10	2	5
Do.....	.25	10	2	5
Do.....	.5	10	2	5
Do.....	1.0	10	0	5
Copper resinate.....	.1	10	1	8
Do.....	.25	10	3	8
Do.....	.5	10	0	8
Mercury stearate.....	.1	10	0	8
Do.....	.25	10	0	7
Do.....	.5	10	2	7
Do.....	1.0	10	5	3
Control.....	—	10	0	7
Lead stearate.....	.1	10	1	8
Do.....	.25	10	1	8
Do.....	.5	10	0	8
Do.....	1.0	10	1	8

SUMMARY

In a series of experiments, a number of organic compounds developed by the Chemical Warfare Service were tested both as stomach poisons and contact insecticides, on third-grown to half-grown eastern tent caterpillars (*Malacosoma americana* Fab.).

The effect of the insecticide upon the host plant was determined by treating bean plants with the material to be tested. Most of the compounds that were toxic to insects proved to be so toxic to the plants as to have no practical value.

As a stomach poison, diphenylamino arsenious oxide was equal in toxicity to lead arsenate, and it was not injurious to the bean plants. It also has some promise as a contact insecticide.

RELATION OF THE MANNER OF FAILURE TO THE STRUCTURE OF WOOD UNDER COMPRESSION PARALLEL TO THE GRAIN¹

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PURPOSE OF STUDY

It is frequently noted in pieces of wood which have failed in compression parallel to the grain, that the line of failure is more or less inclined on the tangential or flat-grain faces but is practically at right angles to the grain on the radial or edge-grain faces. (See figs. 5 and 6.) This seems to be true for a great many species of both hardwoods and softwoods. Evidently there is something about the structure of the wood which tends to cause the failure to be inclined in the one direction. That the plane of failure would be inclined in one direction or another might be expected, since experiments show that materials which, like wood, are neither fully plastic nor easily pulverizable fail along inclined planes when subjected to compression, the angle of rupture being such as to cause failure under the least load necessary to effect a change. The purpose of this study was to determine what there is about the arrangement of the various tissues of wood, or the structure of the individual cells, which almost constantly produces an inclination of the plane of failure in the tangential direction.

MATERIAL

The material for study was chosen from both softwoods and hardwoods available at the Forest Products Laboratory, Madison, Wis. It consisted of 88 pieces of southern yellow pine, 136 of Douglas fir, 100 of Sitka spruce, 110 of oak, 25 of ash, 16 of red gum, 15 of mahogany, 14 of sugar maple, 10 of birch, and 10 of balsa (an exceedingly soft and light tropical wood).

The test pieces had, in most cases, the standard size of 2 by 2 by 8 inches. Some, however, were cut with different cross-sectional dimensions, to see if that made any difference in the manner of failure—with negative results. All specimens were subjected to compression in testing machines according to the standard procedure at the Forest Products Laboratory.

The specimens were in an air-dry condition, except for some of the Douglas fir specimens, which were well above the fiber-saturation point. The density and the maximum crushing strength developed are on record for nearly all.

¹ Received for publication Nov. 21, 1925; issued July, 1926. These investigations were carried on under the direction of Arthur Koehler, in charge, Office of Wood Technology, Forest Products Laboratory, Forest Service, United States Department of Agriculture, Madison, Wis.

Specimens which did not present strictly radial and tangential surfaces, as tested, were afterwards split along the radius and tangent of the annual rings. A surface slightly divergent from the radius might show an inclined failure on the presumably radial surface,

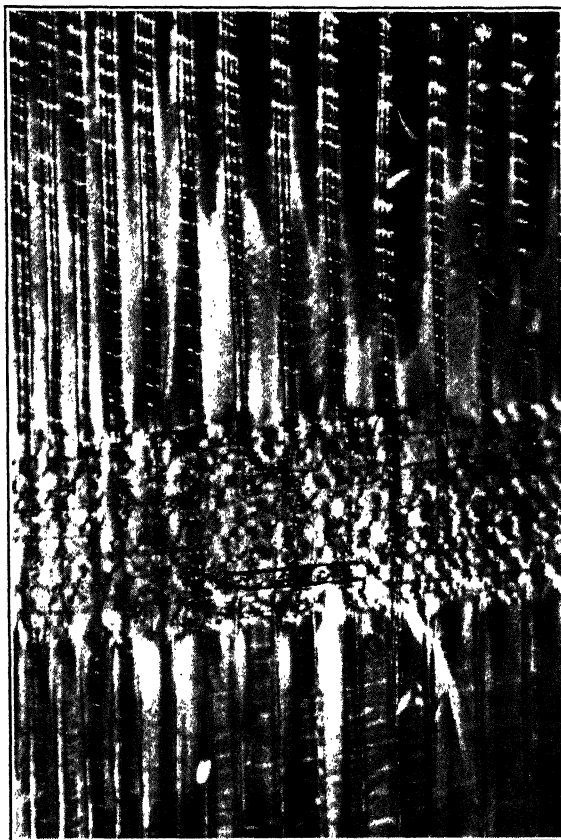


FIG. 1.—Slip planes in fiber walls of Sitka spruce. (The transverse band near the middle is a medullary ray.) Photographed by polarized light. $\times 156$

whereas when the block was split exactly radially the failure appeared practically at right angles to the fibers on the split surface, thus conforming with the basic premise of the investigation.

Longitudinal microscopic sections, including failures in various stages of development, were prepared from many of the test pieces, and different stains were used to bring out contrast. Soon, however, the examination was performed almost entirely with the help of polarized light, which was particularly useful as a rapid and vivid means of discovering the existence of initial failures and of even more minute changes in the cell wall.

SIGNIFICANCE OF SLIP-PLANES IN THE FIBERS

Studying the character of the gross failures in radial and tangential sections with the question in mind, "How and from what do they originate?" one naturally goes to the earliest stages of failure. Immediately one's attention is drawn to the fact that the cell walls show changes attributable to stress, even before a real failure can be considered to be present; and from that observation the necessity of studying the initial changes is obvious.

Robinson² observed what appear to be the preliminary stages of failure in compression along the grain, to which he gave the name of

² ROBINSON, W. THE MICROSCOPICAL FEATURES OF MECHANICAL STRAINS IN TIMBER AND THE BEARING OF THESE ON THE STRUCTURE OF THE CELL-WALL IN PLANTS. Roy. Soc. [London] Phil. Trans. (B) 210: 57. 1920.

"slip lines" or "slip planes." As seen in conifers, he defined them as "the changes that lead to deformation and consist in appearance of extremely fine but sharply defined cracklike lines in the walls of the tracheids."

Slip planes corresponding to those described and illustrated by Robinson can be seen with extraordinary clearness in the fiber walls shown in Figure 1, which represents a radial section of Sitka spruce photographed by polarized light. In the cell walls cut through lengthwise (upper part of figure) each slip plane appears as a short bright line extending across the wall at an angle of about 70° with the longitudinal axis of the fiber. The slope may be in either direction, though only one is clearly shown here, and occasionally the slip planes may cross each other, forming X-shaped hatchings. In the lower part of Figure 1 a surface view of the cell walls shows the slip planes extending around the fiber at a slight incline.

The extraordinary brightness of the slip planes in Figure 1 may be due either to the relative thinness of the section from which the photograph was made or to the fact that the stick from which the section was cut had been subjected to tension after compression, for wood so tested often reveals the slip planes with unusual brightness. A probable explanation of the latter effect is offered in the third footnote.³

There seems to be a wide variation in the brightness as well as the number of slip planes in woods of different species. In general it can be said that the thicker the cell walls and the thinner the sections the more readily they can be observed.

Slip planes are considered the first indication that the wood has been subjected to severe longitudinal compression, either artificially or naturally, for the following reasons:

1. They are much more numerous in parts of cell walls which have actually failed in compression than in other parts. (See fig. 2.)
2. They often are associated with a little displacement or offset of the fiber wall. There is a noticeable similarity between this kind of displacement and the gross displacement of the whole test piece when it has finally given way under compression. The presence of slip planes is strong presumptive evidence that the individual fiber has been injured—that something has given way locally.³
3. The slip planes take up stains selectively just as do the larger injuries to the cell walls.
4. Slip planes are common in many woods, whether intentionally subjected to stresses or not, as has been found from the study of microscopic sections taken from both hardwoods and softwoods. When present in wood not previously subjected to any testing, they are probably to be considered as due to stresses resulting from the weight of the tree, wind action, felling or rough handling, or uneven longitudinal shrinkage. That the last-named is not necessarily the cause, however, was shown by the presence of slip planes in sections

³ It may be for this reason that slip planes have been found to be exceptionally bright (under polarized light) in sections cut from pieces subjected to tension. Under tension, naturally, the injured parts are stretched, thus forming a broader zone which makes them more conspicuous.

Contrary to Robinson's opinion, however, it is doubtful that slip planes are produced in tension parallel to the grain. Their presence in specimens subjected to tensile stresses does not prove that they were formed while those stresses were operative. The fact that slip planes were found to be no more numerous in the region of failure in specimens tested in tension than in wood not artificially stressed indicates that they do not develop in tension.

cut from green wood and mounted for observation in water.⁴ That severe compression stresses may occur in wood before use or test is shown by the advanced compression failures which have been found in new lumber. This phenomenon is by no means very exceptional and has been recognized again and again.⁵

5. To make certain that the slip planes were not formed by the shearing action of the knife in cutting the longitudinal sections of the wood parallel with the fibers, sections were cut with the knife passing through the wood at right angles to the fibers. This did not seem to alter in any way the number of slip planes present. Furthermore, thin and thick sections were cut, and a sharp knife and a dull knife were used, but no difference in the abundance of slip planes was observed. Even more conclusive evidence that slip

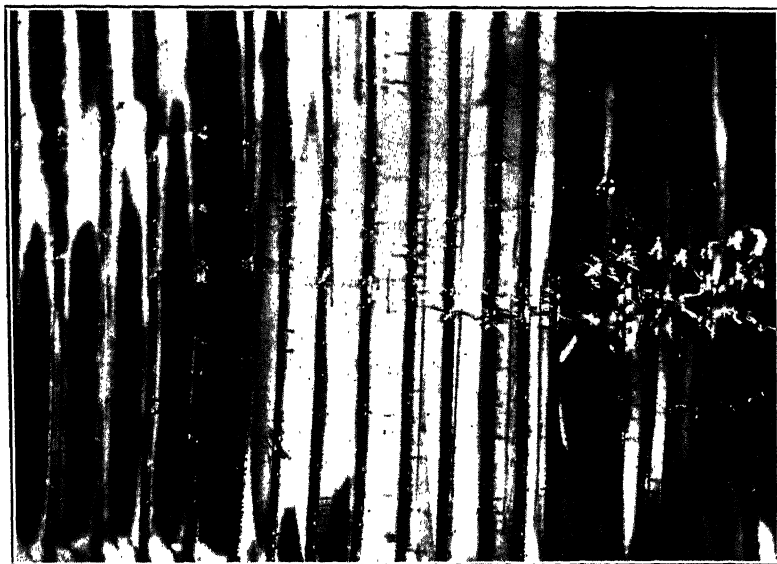


FIG. 2.—Initial compression failure made up of numerous slip planes in Sitka spruce. Photographed by polarized light. $\times 148$

planes are not necessarily due to the shearing action of the knife in making microscopic sections is derived from their presence in carefully macerated fibers from both hardwoods and softwoods. In macerated fibers the number of slip planes may vary considerably. As a rule they are not very numerous or very bright, but they have always been found.

FORMATION OF INITIAL COMPRESSION FAILURES

The next stage after the development of slip planes in compression parallel to the grain of wood is the formation of a localized injury,

⁴ Green material was also studied to determine whether the refractive qualities of wood are changed in consequence of the treatment ordinarily given to the pieces before sectioning. The treatment consisted in boiling the blocks in water to exclude the air, then placing them in hydrofluoric acid to soften them, after which the acid was boiled out again in water and the blocks were preserved in a mixture of water, glycerin, and alcohol. Microscopic sections cut from these blocks and mounted in water showed no difference in refractive qualities under polarized light as compared with material treated in the ordinary way, nor was an appreciable difference in either the number or the character of the slip planes noted.

⁵ KOEHLER, A. SELECTING WOOD FOR AIRPLANES. *Sci. Amer. Sup.* 88: 148-149, illus. 1919.

MARKWARDT, L. J. COMPRESSION FAILURES AS DEFECTS. *Hardwood Rec.* 39: 24-25, illus. 1914.

extending in a more or less definite line or zone across a number of adjacent cells. It can readily be recognized as the first definite indication of failure, and for that reason it is termed the initial failure.⁶ It can not be seen without a microscope, however, and is best seen with a polarizing microscope.

This stage, and the gradual changes leading up to it, are illustrated in Figure 2. Slip planes are scattered throughout the wood, as the illustration shows, although they do not show so clearly as in Figure 1. At the extreme left of the illustration they can be seen connecting up into definite lines, which become more numerous and pronounced toward the right, especially in the summer wood. The slip planes probably had already become numerous before the initial compression failure had formed. Then they became linked up by the formation of others, producing a distinct zone of failure.

From a number of observations it can be said that the initial failure in both radial and tangential sections may extend either horizontally or in an inclined direction across the fibers—more often the latter, especially in the summer wood. At this stage no actual bending or buckling of the fibers has taken place. There is not yet anything more than the slight offset in connection with the slip planes in the individual fibers, as already described, but they are numerous and close together.

Compression failures, even when not readily apparent to the eye, should always be regarded as a source of weakness, and material in which they are present should not be used where strength is of prime importance. Under slowly applied loads they do not greatly affect the strength in compression parallel to grain, although they have a marked influence on bending properties, particularly when present on the tension side of a member. The shock-resisting ability of wood containing compression failures is even more seriously affected, complete failure of the member usually occurring along the injured section with little deformation and very suddenly. For these reasons alone it is easily seen that microscopy by polarized light is an invaluable as well as an easy method of detecting very early stages of injury or failure.

DEVELOPMENT AND NATURE OF GROSS FAILURES

To study the development of gross failures in wood subjected to end compression, radial and tangential sections were prepared. Originally the sections were stained, since the area of failure for some reason takes certain stains⁷ selectively and thus stands out from the rest of the field. But staining, however helpful it may be, is not so good as using polarized light, which, as in the case of initial failures, is sometimes almost indispensable. Under polarized light the failures stand out bright against a darker background, or vice versa. The contrast brings out every detail necessary for study in stained as well as in unstained sections. The difference in the brightness of the injured and uninjured parts is due partly to local displacements of the cell-wall material, and partly to the fact that

⁶ Slip planes are of too common occurrence and usually too scattered in wood to be considered definite regions of failure.

⁷ Potassium ferrocyanide, to which ferric chloride was added, was used, this giving the so-called Berlin-blue reaction. Another satisfactory stain is picro-aniline blue.

in the area forming the failure the slip-planes are so numerous and close together that the whole cell wall seems to be changed in optical properties.

Observations on microscopic sections indicate that when a certain part of the wood under stress contains a number of initial failures which have developed to a considerable extent, that part is weakened and thus forms an appropriate place for a gross failure to begin. The gross failure seems to start along, and to include, some of the initial failures, but its development is not always guided by the initial failures, for these are not continuous as a rule, whereas the gross failure is more or less so.*

GROSS FAILURES DUE TO BUCKLING OF CELL WALLS

The grosser failures which are distinct to the naked eye can be seen under the microscope to be due to buckling of the cell walls. The final extent of the buckling is dependent on how far the compression is carried, i. e., how long the increasing load is allowed to act on the test piece after the elastic limit has been passed.

It is necessary to distinguish here between buckling and crinkling, because the occurrence of these two types of failure varies according to the wood tested and also between the spring-wood and summer-wood parts of a given piece.

By buckling is understood the bending of the fibers under stress, resulting in such a deformation of the cell wall that the parts originally in one axial line are no longer in that position, but, while remaining parallel to one another, have become displaced by a certain (perhaps small) distance, the actual "buckle" forming the connection between the two parts. The deformation, or buckle, is hereafter referred to as an "offset." It is to be distinguished from the offset previously described in connection with slip planes.

Crinkling results in a permanent deformation (telescoping) in the part that crinkles, but the parts of the fibers above and below remain in the same line or almost so.

The terms "buckling" and "crinkling" are used in the same sense by Robinson.⁸ They are respectively equivalent to the terms "bending" and "buckling" used by Brush.⁹

In conifers an obvious difference between the spring-wood cells and the summer-wood cells is that the latter, being smaller and thicker walled, are more likely to buckle, while the former, being thinner walled, show more or less indefinite patterns of crinkling in the early stages of the gross failure. Later, of course, as the buckling of the summer wood progresses, the spring wood also buckles so as to adapt itself to the contour. It is quite evident that in all such cases it is the summer wood that carries most of the compressive load applied parallel to the grain, as might be expected in woods which have pronounced summer wood. When there is no such pronounced difference between summer wood and spring wood—as in birch, maple, and red gum, for example—the material of the various cells more uniformly share the load and the contrast between buckling and crinkling is not so apparent. Over against these cases of wood of almost entire "summer-woodlike" structures,

⁸ ROBINSON, W. Op. cit.

⁹ BRUSH, W. D. A MICROSCOPIC STUDY OF THE MECHANICAL FAILURE OF WOOD. U. S. Dept. Agr., Forest Serv., Rev. Forest Serv. Invest. 2: 33-38, illus. 1913.

in which all the cell walls in the regions of failure buckle, stands the example of balsa, on which a few tests have been made. Here the phenomenon of failure could probably better be described as crinkling only, the whole structure being more of the nature of spring wood.

It is the direction in which the offset due to buckling takes place which predetermines the direction in which the region of failure is inclined. If the buckling takes place in a tangential direction, as it nearly always does, then the failure will be inclined on the tangential face. (See fig. 3.) For it is obvious that the least amount of friction and shearing will take place between fibers in buckling when their curvatures fit together most closely, a condition which can occur only when the curvature of one fiber is a little below that of the adjacent one. Hence the zone of a series of such curvatures would assume an angle with the horizontal.

Radial offsets have been found in a few cases in the softwoods, but even then it was in only a very few that the gross failure formed an angle of considerably less than 90° with the vertical on the radial face. Figure 4 illustrates one of the cases with a definitely radial offset,

but with the gross failure still almost horizontal, or at an angle of about 90° with the grain. It is noticeable, however, that within each of the summer-wood layers (the lighter-colored bands in the figure) the failure is decidedly at an acute angle. Thus it is possible, evidently, that numerous little parts of the whole failure may follow their own direction over a certain distance, then merge again into the main trend of the failure of which they are parts.



FIG. 3.—Tangential offset in gross compression failures in Douglas fir. Photographed by polarized light. $\times 30$

RÉSUMÉ OF COMPRESSION TESTS

In the entire lot of 324 softwood specimens tested in end compression, only three cases were found in which the failure made any considerable angle with the horizontal on the radial faces, and in the 200 hardwood specimens tested none was found. In the three exceptional cases the angle was much smaller than those usually seen on tangential faces. Only one of the three showed a strictly radial offset, and its abnormal deformation may possibly be explained by the presence of a small knot in the zone of failure. The other two had both tangential and radial offsets. In these two, as well as in eight others which had both tangential and radial offsets, but horizontal failures on radial faces, the radial offsets may have been due to the advanced stage to which the failures were carried in the testing machine.



FIG. 4.—Radial offset (unusual) in gross compression failure in Douglas fir. Photographed by polarized light. $\times 28.5$

According to Robinson¹⁰ the behavior of spruce is exceptional, in that it forms a radial offset as a rule. Such a finding was not confirmed here. Of 100 standard-sized pieces of Sitka spruce that were tested in compression parallel to the grain, by far the greater number had a quite normal tangential offset, only three cases showing a composite radial and tangential offset. (These are included in the 10 referred to above.) That either a radial offset or an inclined gross failure on the radial face is quite exceptional in all the woods studied, including Sitka spruce, seems conclusively demonstrated.

Considering a possible relation between the angle of gross failure and density, Thil¹¹ states that the harder (heavier) the wood, the smaller the angle the failure makes with the vertical.

¹⁰ ROBINSON, W. Op. cit., p. 52, 53, 56.

¹¹ THIL, A. CONSTITUTION ANATOMIQUE DU BOIS.—ETUDE SUR LES FRACTURES DES BOIS DANS LES ESSAIS DE RÉSISTANCE. Publié par commission des méthodes d'essai des matériaux de construction sous les auspices du Ministère des travaux publics de France. Tome III, rapports particuliers, p. 140-141. Paris. 1900. [Not seen.]

The general angle of the gross failure with the vertical on strictly tangential faces showed the following average values in the softwood and oak specimens tested for this study:

	Degrees
Sitka spruce (dry)-----	70
Douglas fir (dry)-----	64½
Douglas fir (moist)-----	63
Southern yellow pine (dry)-----	59½
White oak group (dry)-----	57

The number of tests of hardwoods other than oak was not large enough to afford a fair average. The results bear out Thil's statement, since the angle is largest in Sitka spruce and smallest in oak, with Douglas fir and yellow pine intermediate, all inversely as the density of the species. The plane of failure was somewhat more definitely marked, and the buckling more gradual, in the harder woods.

For the southern yellow pine, Douglas fir, and oak used in this study, the angle of the gross failure with the vertical was plotted against the specific gravity of each specimen. In oak, especially, the different pieces were of widely different specific gravities, so that the mode of variation could be observed over a fairly representative range. From the diagrams it was concluded that *within a species* there is no controlling relation between the angle of failure and the specific gravity.¹²

SUPPLEMENTARY TESTS TO DETERMINE EFFECT OF CROSS SECTION

In order to determine whether the relative dimensions of the cross section of rectangular specimens have any influence on the manner of failure, provided the axial length of the test piece is such that bending is practically excluded, a series of tests was performed on red gum, mahogany, ash, Douglas fir, and southern yellow pine. For each species test pieces of the following transverse dimensions, in inches, were prepared: 1¼ by 2½, 1¼ by 1¾, 1¼ by 1½, 1¼ by 1¼, and 1¼ by ⅝. The length of each piece was 4 inches. Figures 5 and 6 show the red-gum specimens after test, with moisture sections cut off and placed above the pieces, showing the direction of the growth rings. Figures 5 and 6 show that in all cases, no matter what the dimensions of the transverse section, the blocks have a tangential offset; the failure makes a definite acute angle with the grain on the tangential face; and on the radial face the failure runs across horizontally, or almost so, all in the regular manner.

DISCUSSION OF THE MODE OF FAILURE

Many reasons have been advanced by different investigators in an endeavor to explain the typical inclination of the gross failures on tangential faces. Thil¹³ attempted to show that the angle of the

¹² P. Jaccard makes the same statement, "qu'il n'existe pas de type de rupture spécifique, c'est-à-dire propre à chaque espèce ligneuse . . ." JACCARD, P. ETUDE ANATOMIQUE DE BOIS COMPRIMÉS. Mitt. Schweiz. Centralanst. Forstl. Versuchsw. 10: 57. 1910.

¹³ THIL, A. Op. cit.

failures is related to a supposed spiral arrangement of the medullary rays. Supposing the tops of the rays to be naturally arranged along imaginary spirals around the stem, he considered the spirals as lines of least resistance. A careful examination of tangential sections shows, however, that such an arrangement of rays evidently does not exist. This observation is confirmed by Jaccard's remarks upon Thil's article: "One needs only to reproduce by the camera lucida or by photomicrography the distribution of rays on the tangential faces of the test pieces which have been compressed, to be convinced that no constant relation exists between such distribution and the direction of the lines of rupture." The 15 tests made in the present investigation on mahogany (in which the rays are in horizontal rows) gave consistent evidence against Thil's hypothesis, since they produced the same type of offset and inclined failure on the tangential faces as is typical of other woods.

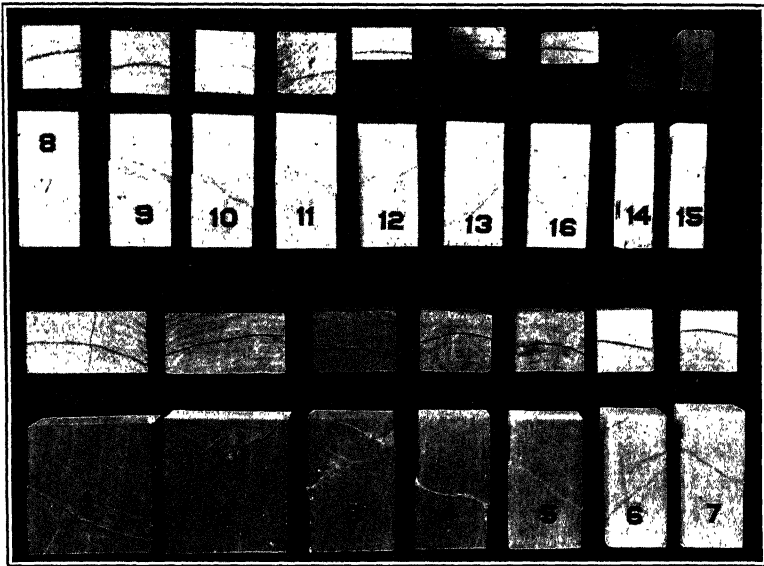


FIG. 5.—Tangential surfaces of red gum, a homogeneous wood, showing inclined failures due to compression parallel to the grain. (The pencil lines across the end surfaces of sections above each specimen indicate the direction of the annual rings)

A somewhat similar arrangement to that supposed by Thil for the rays could be imagined for the wood fibers in a tangential plane in contrast to their frequent horizontal arrangement in radial planes; but here also a study of tangential sections affords no evidence of the fibers being arranged in inclined zones corresponding to the zones of failure.

The fact that in the gross compression failure on the tangential face a separation sometimes is found between the rays and the surrounding fibers probably accounts for the supposition that the junction of ray and fiber forms a weak spot which could therefore be considered as the starting point of the failure. But closer microscopic examination shows that this separation at the rays takes place only after buckling of the fiber material as a whole has progressed

considerably, at which stage the fibers tend to separate from each other as well. Figure 3 shows that in the case of advanced buckling fibers may separate, even when there are no rays in the immediate neighborhood. Moreover, in the comparatively rare cases of radial offsets, it is known that separation of fibers may occur in the radial direction, where the rays could have no weakening influence. In fact, the first steps in the buckling of the fibers take place on too small a scale to be affected by the rays, and manifest no relation to them. It has already been explained¹⁴ in this paper that the origin of failure lies in the giving way of the individual cell wall, and the development of these minute injuries into the later failures has been indicated.

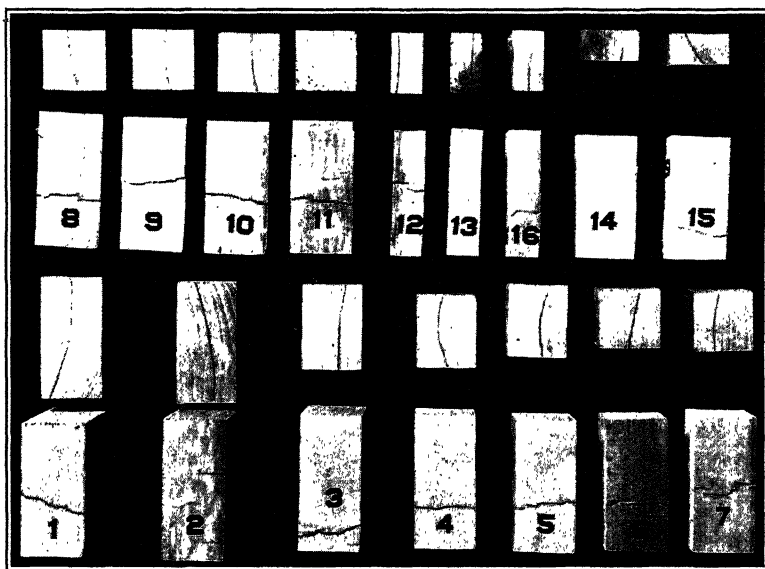


FIG. 6.—Radial surfaces of same blocks as in Figure 5, showing more or less horizontal conformation of failures

Jaccard¹⁵ holds that rupture is determined by points of least resistance in the wood¹⁶ which reduce cohesion and the homogeneity of the wood fibers and which favor their bending and folding. According to Jaccard, the pits in the walls of the tracheids or fibers form such points of weakness. But again the photomicrographs made for the study of initial failures show that evidence for supposing such a relation is lacking, and the argument therefore fails as an explanation of the character or the commencement of the failure.

That nonhomogeneity arising from any structural differences as between spring wood and summer wood is not to be regarded as the explanation of the inclination of the plane of failure in wood subjected to compression is shown by the fact that woods of highly homogeneous structure in this respect—for example, red gum, birch, maple, and

¹⁴ As has also been shown by ROBINSON, W. Op. cit.

¹⁵ JACCARD, P. Op. cit.

¹⁶ In the present paper only perfectly clear material is considered. Jaccard includes the following among his "points of least resistance": "Knots, of which some are very small and do not even appear on the surface of the specimen, by which the straightness of the fibers is markedly altered and their cohesion interrupted."

mahogany—behave in exactly the same manner as less homogeneous woods such as oak, ash, Douglas fir, and yellow pine.

The only remaining known and consistent factor which could possibly exert sufficient influence to account for such almost universal inclination of the failure in a tangential direction must be looked for in the action of the medullary rays. It has already been pointed out that it is neither the spiral arrangement of the rays nor a weak bond between the rays and the wood fibers that can account for the tangential inclination of the failure. It therefore seems reasonable to assume that the cause must lie in the stiffening effect which the rays have radially, this increasing the resistance of the fibers to buckling in that direction and causing them to buckle tangentially, which necessitates the inclination of the failure on the tangential face. This conclusion can, of course, be arrived at only by the process of disproving the possible influence of other factors. It can not be proved directly, since the same piece of wood can not be tested with and without rays.

CONCLUSIONS

This study corroborates Robinson's statement that slip lines, or slip planes, are the first indication of injury in compression parallel to the grain, but it is doubtful whether they are likewise produced in tension along the grain.

The early or initial failures, consisting principally of numerous localized slip planes, show no such distinctive difference between their radial and tangential aspects as that exhibited by gross failures. Apparently, however, they are sources of zones of weakness within which the buckling of the fibers resulting in gross failures may start more easily than elsewhere.

The gross failure in compression parallel to the grain consists of a buckling of the fibers. The least amount of friction between fibers occurs when the plane of buckling, or failure, is inclined. The inclination of the failure occurs, as a rule, in a tangential direction. The present writer has shown that the only probable explanation of this behaviour is that the medullary rays increase the resistance to buckling in a radial direction, thus causing the line of failure to be inclined on the tangential surface.

THE IMPORTANCE OF CLEARING THE HYDROLYZED SOLUTION IN THE DETERMINATION OF ACID-HYDROLYZABLE CARBOHYDRATES IN GREEN PLANT TISSUE¹

By V. H. MORRIS, *Assistant Agronomist (Plant Chemistry)*, and F. A. WELTON, *Associate Agronomist, Ohio Agricultural Experiment Station*

INTRODUCTION

While making a study of the available literature bearing on the separation and quantitative estimation of the various carbohydrate materials in plant tissue, preparatory to some experimental work, it became apparent that all workers do not follow the same procedure in the manipulation of some of the methods involved.

One such method is the determination of starch and similar substances by weak-acid hydrolysis. In this process some workers omit the step of clearing the solution containing the hydrolyzed material, and others apparently consider it necessary. The more generally accepted method appears to be that of clearing the solution by adding an excess of a solution of either basic or neutral lead acetate solution, deleading with sodium phosphate or some other salt solution, and either filtering the solution or allowing the precipitate to settle overnight before taking an aliquot for the determination of the reducing power.

OBJECT

When many samples are to be analyzed, it is desirable to eliminate unnecessary steps. In the work undertaken there were nearly 200 of the alcohol-preserved samples of green tissue to be analyzed. In view of the large amount of time and labor required to clear the hydrolyzed solutions, it was thought advisable to make a preliminary study of this step, to the end that the process might be shortened or perhaps eliminated altogether.

MATERIAL

Among the samples preserved for carbohydrate analysis, eight different kinds of green plant material were represented as follows: Wheat culms, oat culms, blue grass, soy-bean stems, cornstalks, Canada-thistle roots, red-clover roots, and wheat rhizomes. The wheat and oat culm samples were taken at heading time. The blue grass was fresh pasture about 5 inches high. The soy beans were sampled when in bloom. The cornstalks were taken when at about the earing stage. The thistle roots, clover roots, and wheat rhizomes were dug up late in the fall. Two samples of each class of material grown under different environmental conditions were selected so that the results of both samples could be considered fairly typical for that class.

¹ Received for publication Dec. 3, 1925; issued July, 1926. Published with the approval of the Director of the Ohio Agricultural Experiment Station.

METHODS

PRESERVATION

In most cases 100 grams of the green tissue was taken for the sample, and after being cut into small pieces $\frac{1}{2}$ to 1 inch long the sample was placed in alcohol of such a strength that the final preserving medium would be 70 per cent, and the whole was heated in a water bath at 78° C. for one hour.

EXTRACTION

The samples were extracted with boiling 80 per cent alcohol in a large Soxhlet extractor for 8 to 12 hours, after which the residue was dried on a steam plate for several days, then placed in a vacuum oven at 105° C. for 8 hours, and then ground finely.

HYDROLYSIS

For hydrolysis, 3-gram samples were taken of wheat culms, oat culms, blue grass, and soy-bean stems; 2 grams each of cornstalks, thistle roots, and clover roots; and 1 gram of wheat rhizomes. The samples were weighed into 350 c. c. Kjeldahl flasks, 200 c. c. of 2.5 per cent HCl was added, and the flasks, with small funnels in the necks, were placed in a boiling water bath for two hours. The solutions were then filtered through Gooch crucibles with linen filters, washed well with hot water, and then the filtrates were transferred to 500 c. c. volumetric flasks and made up to volume. Aliquots of 100 c. c. each were transferred to 250 c. c. volumetric flasks, cleared or left untreated, made up to volume, and shaken.

DETERMINATION OF REDUCING POWER

For the determination of reducing power, a modification of the method of Quisumbing and Thomas² was used. A 25 c. c. aliquot of the sugar solution was transferred to a 100 c. c. centrifuge tube, 60 c. c. of Fehling's solution was added, and the tube was heated in an electric water bath for 30 minutes at 80° C. The tube was then centrifuged for 10 minutes, the supernatant liquid was poured off, and the reduced copper was shaken with about 15 c. c. freshly boiled distilled water, and again centrifuged for 10 minutes. The supernatant liquid was again poured off, and the reduced copper was determined by the Bertrand method, titrating with N/20 KMnO_4 .

EXPERIMENTAL DATA

A preliminary test was carried out with two samples of wheat culms which had exactly the same amount of polysaccharides present. The generally accepted method of clearing described previously was followed, an excess of neutral lead acetate being added, then delead with Na_2HPO_4 and allowed to settle overnight. The treatment and results are given in Table 1. The results in all tables are expressed in cubic centimeters of N/20 KMnO_4 necessary to titrate the reduced copper.

² QUISUMBING, F. A., and THOMAS, A. W. CONDITIONS AFFECTING THE QUANTITATIVE DETERMINATION OF REDUCING SUGARS BY FEHLING SOLUTION. *Jour. Amer. Chem. Soc.* 43: 1503-1526, illus. 1921.

TABLE 1.—*Comparison of cleared and uncleared aliquots*

Sample	Unneutralized, uncleared	Neutralized, uncleared	Neutralized, cleared
1	6.4	6.3	6.2
2	6.3	6.4	6.3

The deleading process is the one that takes the most time, so it was decided to determine whether the lead acetate could be added drop by drop until complete precipitation and coagulation was obtained, without adding any excess. Since the point of such complete precipitation is not very sharp, an additional amount of 1 c. c. of the lead solution was added to a second aliquot without subsequent deleading. The results of this procedure are given in Table 2.

TABLE 2.—*Effect of clearing without excess of lead solution*

Sample	Unneutralized, uncleared	Neutralized, plus lead acetate to cause coagulation	Neutralized, plus 1 c. c. excess lead acetate
3	4.8	4.6	4.5

Apparently this method of clearing does not give results materially different from the check; in fact, an excess of the lead solution may be added without affecting the results.

A group of four samples of different plant tissues was then used in further testing this method of clearing, adding the lead acetate drop by drop until no more precipitation was observed. The results are given in Table 3.

TABLE 3.—*Further test of clearing without excess of lead solution*

Sample	Unneutralized, uncleared	Neutralized, cleared without excess of lead solution
Soy beans.....	8.9	8.9
Thistle roots, No. 1.....	17.1	17.4
Thistle roots, No. 2.....	15.1	15.3
Clover roots.....	13.1	13.4

In three tables given thus far, there is no significant difference between the check aliquot (that left unneutralized and uncleared) and the cleared aliquot.

In order to test this point further, and also to determine whether there was any difference between different kinds of plant material with respect to the necessity for clearing the hydrolyzed solution, eight classes of plant tissue, including the two different samples of each class, were hydrolyzed. The treatment of the different aliquots and the results are given in Table 4.

TABLE 4.—Comparison of eight kinds of plant tissue with respect to clearing the hydrolyzed solution

Sample	Laboratory No.	Unneutralized, uncleared	Neutralized, uncleared	Neutralized, cleared without excess	Neutralized, cleared with excess lead plus PO_4
Wheat culms.....	238	8.1	8.3	8.3	10.1
Do.....	239	10.0	10.1	10.0	8.0
Oat culms.....	274	8.3	8.3	8.2	8.4
Do.....	275	8.4	8.2	8.2	11.1
Blue grass.....	328	11.7	11.7	11.4	8.4
Do.....	329	8.5	8.5	8.5	8.1
Soy-bean stems.....	349	8.2	8.2	8.0	8.8
Do.....	350	8.8	8.8	8.7	7.9
Cornstalks.....	358	8.2	7.5	7.8	7.5
Do.....	360	7.7	7.7	7.7	18.7
Canada-thistle roots.....	363	19.3	18.7	18.9	17.4
Do.....	364	17.7	17.4	17.1	15.3
Red-clover roots.....	371	15.8	15.8	15.4	14.9
Do.....	372	15.4	15.0	15.0	6.2
Wheat rhizomes.....	379	6.2	6.2	6.2	5.5
Do.....	380	5.7	5.9	5.5	

With a very few exceptions, apparently the same result is obtained whether the hydrolyzed solution is cleared or not, and, if cleared, it is not necessary to add an excess of the lead solution and then delead. Low or high results on one aliquot out of the four of each sample were obtained in four cases. These results were obtained each with a different treatment, and in every case the other three aliquots checked among themselves, indicating that probably the error was due to some step in the procedure, such as insufficient shaking of the flask after making up to volume, rather than to the effect of the treatment.

EFFECT OF REACTION OF SOLUTION

The importance of the reaction of the hydrolyzed solution when the lead acetate solution is added has not been very strongly emphasized in published methods. It is very important that the reaction of the solution be kept on the acid side. The addition of lead acetate to a neutral solution results in shifting to an acid reaction, since lead acetate is an acid salt. On the other hand, the addition of a fairly strong basic salt like Na_2PO_4 in deleading changes the reaction to alkaline while there is still an excess of lead.

The effect of the alkaline reaction obtained in this way is shown in Table 5, in which the results obtained with several of the samples, keeping one aliquot of the solution acid, are compared with another aliquot of the same solution allowed to become alkaline as suggested above. The solutions were all cleared with lead acetate, adding an excess and deleading with the phosphate solution.

TABLE 5.—*Effect of reaction of hydrolyzed solution*

Sample	Laboratory No.	Cleared with excess lead, acid reaction	Cleared with excess lead, alkaline reaction
Wheat culms.....	239	10.1	8.6
Oat culms.....	274	8.0	6.8
Do.....	275	8.4	6.8
Blue grass.....	328	11.1	10.5
Do.....	329	8.4	7.8
Soy-bean stems.....	350	8.8	8.4
Cornstalks.....	358	7.9	7.6
Do.....	360	7.5	7.2
Canada-thistle roots.....	363	18.7	16.9
Do.....	364	17.4	16.2
Red-clover roots.....	371	15.3	14.9
Do.....	372	14.9	13.9

DISCUSSION

The advantages of being able to determine, without previous clearing, the reducing power of the solution obtained in the determination of the weak-acid-hydrolyzable carbohydrates, are very obvious. The saving in time and labor is considerable.

The only apparent disadvantage of this procedure is that with some plant materials the precipitate of cuprous oxide obtained is yellowish brown and is in a more finely divided state. The use of the centrifuge in determining the amount of the oxide has a decided advantage over any filtration procedure, on account of the danger of some of the more finely divided particles passing through the filtering medium. The size of the copper particles does not make any real difference in centrifuging, because the smallest crystals are thrown down readily by centrifugal force.

SUMMARY

The data obtained in the experimental work reported in this paper indicate that with alcohol-preserved samples of green plant tissue of the kinds used in this experiment it is unnecessary to clear, or even neutralize, the hydrolyzed solution before determining the reducing power; and that it is essential that the reaction of the hydrolyzed solution be kept on the acid side in the presence of the lead solution.





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A CYTOLOGICAL STUDY OF PUCCINIA TRITICINA PHYSIOLOGIC FORM 11 ON LITTLE CLUB WHEAT¹

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INTRODUCTION

Cytological study of plant diseases brings out facts otherwise inaccessible. A study of the behavior of host and parasite in the infection, the details of their interaction as seen under the microscope, is one avenue of approach to the larger field of immunity.

The present problem was suggested by E. B. Mains, who noted that the susceptibility of Kanred wheat to *Puccinia triticina* varied with age and environment, and thought that a microscopical study of the different types of reaction to the rust might help to explain it. The work was soon broadened to include a study of Little Club, a variety uniformly susceptible to the rust, and Malakoff, a strongly resistant variety. This paper presents that part of the investigation dealing with Little Club.

The leaf rust of wheat (*Puccinia triticina* Erikss.) was formerly included with other species under the name of *Puccinia rubigo-vera*. Eriksson (14)³ separated it under the name *Puccinia triticina*. A historical account of the studies of this rust is given by Jackson and Mains (20).

The uredinia and telia are found on wheat. Until recently, the aecial host was unknown in spite of numerous attempts to determine it. The work of Jackson and Mains in 1921 (20) proved that the aecia are borne on species of *Thalictrum* native to Europe and Asia, particularly *T. delavayi* and *T. flavum*.

The bearing of this on the origin of the rust is stated as follows (20, p. 170): "*Puccinia triticina* is considered to be of foreign origin, because wheat, for which it shows close specialization, is an introduced host, and because the most susceptible species of *Thalictrum* which serve as aecial hosts also are exotic." In America, where the aecial hosts are practically absent, the rust probably lives on as a continuous series of uredinial generations.

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² The writer is indebted to C. R. Ball and H. B. Humphrey for suggestions and for a careful reading of this manuscript, to E. B. Mains for suggestions and materials, and to the Divisions of Genetics and Agronomy of the University of California for courtesies extended during the work.

³ Reference is made by number (italic) to "Literature cited," p. 221.

MATERIAL AND METHODS

The strain of leaf rust used in these studies was collected by W. W. Mackie at Eureka, Calif., in the summer of 1923. It was cultured in the greenhouse, a single-spore culture was made, an increased generation was grown from the single uredinium, and the rust was carried on in greenhouse cultures. A specimen sent to E. B. Mains was identified as *Puccinia triticea* physiologic form 11.

The seedlings used for cytological study, Little Club C. I. 4066, were grown and inoculated in the field, the first set in April, 1924, the second in May, 1925. They were covered during the 48 hours following inoculation. The older infected plants studied were grown in the field in the late winter and spring of 1925, and were continuously infected from the seedling stage to maturity.

Material was fixed at intervals from the time of inoculation until the uredinium was old. The fixing fluid was chrom-acetic-urea, of the usual strength or diluted. Material fixed in the cold (40° to 45° F.) proved superior to that fixed at room temperature. The usual methods of dehydration and embedding were followed. Paraffin of 50° C. melting point gave the best results. The triple stain was most satisfactory.

INVESTIGATIONS

PHENOMENA OF ENTRANCE

The seedlings used in the study of entrance phenomena were from seed sown on March 5, 1925, and had encountered rainy weather. The upper leaves (fourth to sixth) were heavily inoculated May 11 during a rain, and were covered to prevent the washing off of the spores.

Many spores germinated during the first night, others during the second, and still others during the third. Freshly formed germ tubes could be found in any lot of material fixed during the first few days.

Infection by urediniospores takes place through the stomata, which, in wheat, are abundant on both surfaces of the leaf. The germ tube grows along the leaf surface to a stoma. Then the protoplasm along the whole length of this tube flows on into the tip, forming there an aggregation called the appressorium. When unobstructed, it rounds up into a hemispherical cushion of rather dense protoplasm just over one end of the stomatal aperture. A typical appressorium is represented in Plate 1, A. The stoma is cut longitudinally, showing one guard cell *a*, with its unevenly thickened walls and its plastids and elongated dumb-bell-shaped nucleus. The appressorium *b* occupies one end of the shallow cavity at the entrance of the stoma. A portion of the empty germ tube is seen at *c*, and a septum isolates it from the appressorium.

EXPLANATORY LEGEND FOR PLATE 1

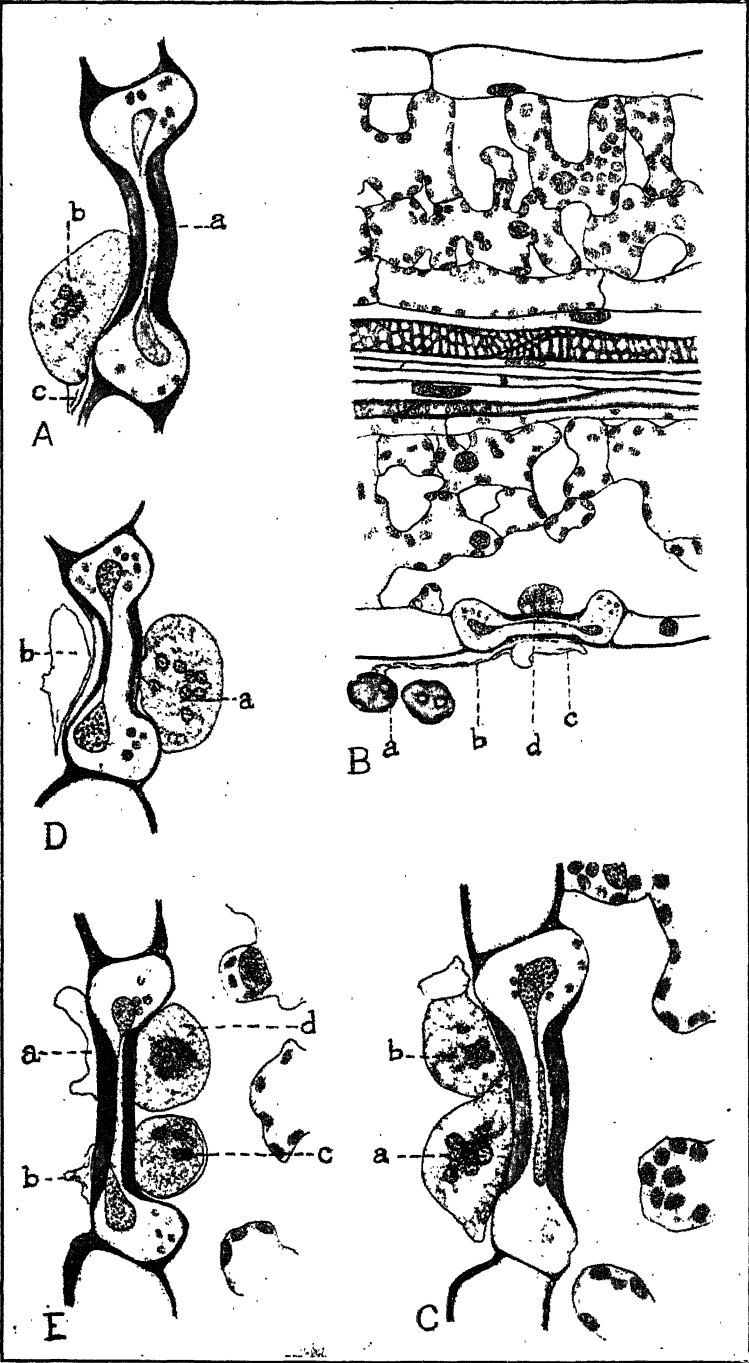
A.—Third day after inoculation. Longitudinal section of stoma bearing appressorium. Guard cell at *a*, remnant of germ tube at *c*, appressorium with 4 nuclei at *b*. $\times 730$

B.—Section through leaf with fungus at stoma. The spores are at *a*, germ tube at *b*, empty appressorium at *c*, and substomatal vesicle at *d*. $\times 333$

C.—Stoma bearing two distinct appressoria, *a* being the older, with *b* partially superposed upon it. $\times 730$

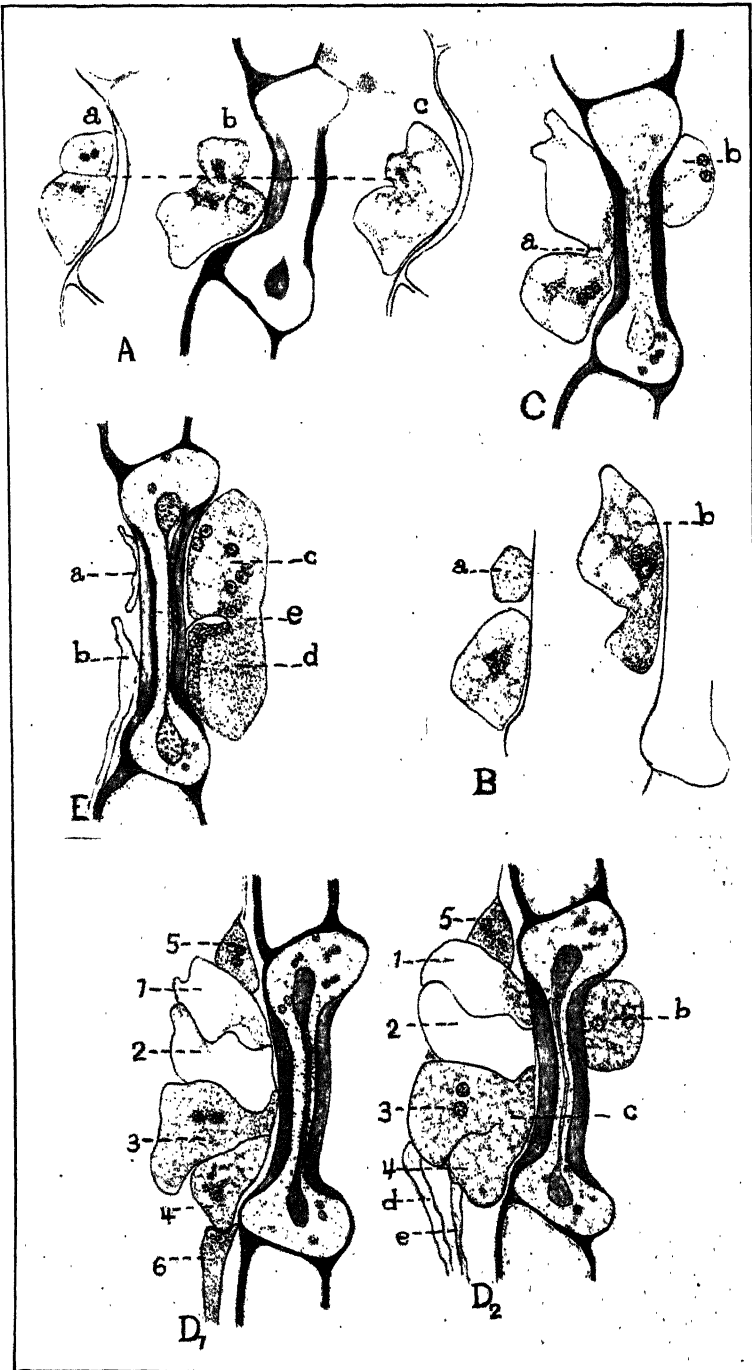
D.—Stoma with the empty appressorium *b* on the outer surface and the substomatal vesicle *a* within. The vesicle contains eight nuclei. $\times 730$

E.—Second day after inoculation. Stoma with two fungi. The appressoria *a* and *b* entered at the ends of the stomatal slit, forming the vesicles *c* and *d* within. One vesicle has four nuclei, the other more. $\times 730$



Puccinia triticea physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 202)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 203)

When, in inoculation, the spores are placed on the upper surface of the leaf, they naturally adhere to the minute parallel ridges running lengthwise on the surface of the leaf. As the longitudinal rows of stomata lie near the base of the little valleys between the ridges on the leaf, the distance the germ tube must grow to its goal may be considerable. The lower surface of the leaf is smoother, and here spores may lodge close to the stoma, and one sometimes sees, as in Plate 1, B, the spore *a*, the germ tube *b*, the appressorium *c*, and the substomatal vesicle *d*, all in the same section.

It is not unusual to find two or more appressoria occupying the same stoma. (Pl. 1, C.) In this case *a* is apparently the older; and *b*, the late comer, is partly superposed on *a*. Fully formed appressoria usually have four nuclei (pl. 1, A and C, at *b*) or sometimes more (pl. 1, C, *a*).

Entrance usually is effected near the end of the stoma. The delicate wall of the appressorium collapses (pl. 1, D, *b*) as its contents pass through the stomatal slit to form the vesicle within. The substomatal vesicle (pl. 1, D, *a*) lies in contact with the inner face of the guard cells. It is an ovoid or irregularly ellipsoid body containing dense cytoplasm and nuclei. When a stoma carries more than one fungus, both may enter. In Plate 1, E, the two collapsed appressoria at *a* and *b* are on the outer surface, and just within are the two separate vesicles, *c* and *d*. The number of nuclei in the vesicle varies. In Plate 1, E, *c*, there are four; in Plate 1, D, *a*, there are eight; in Plate 1, E, *d*, the nuclei form a central cluster so dense that they can not be counted, but there probably are more than eight.

Material was fixed twice a day during the early stages of infection. In the material fixed on the first three mornings, many fungi had just entered the host and formed the substomatal vesicle. (Pl. 1, D and E.) In material fixed in the afternoons the majority of these substomatal vesicles had pushed out the first infecting hypha. (Pl. 3, B.) This daily rhythm in the early stages of infection suggests at least that the time of entrance is conditioned by the daily stomatal movements. It is perhaps indicative that entrance is not effected by mechanical force or by chemical action, but waits upon the natural opening of the stoma. That the fungus, however, does secrete some substance that affects the guard cells seems probable. Its effect is usually very inconspicuous at this stage, but in slightly older infections (pl. 3, C) the guard cell walls are often noticeably altered at the surface of contact with the fungus. Rarely in young infections, but more commonly in older, the stomata occupied by fungi are killed. The connection, if any, between this action on the guard cells and the entry of the fungus is not clear.

Entrance counts were made of fungi fixed in the morning, 40 hours after inoculation. Only 37 out of 204 (about 18 per cent) had not entered.

EXPLANATORY LEGEND FOR PLATE 2

A. Section of dead guard cell bearing an appressorium. Letter drawn at three levels—*a*, *b*, and *c*. At the upper (*a*) it looks like two in contact; at the median (*b*) and the lower level (*c*) the two are connected. $\times 730$

B.—Successive sections of same stoma. Appressoria appear separate in *a* and connected in *b*. $\times 730$

C.—Two appressoria connected at *a*. Single substomatal vesicle forming at *b*. $\times 730$

D₁ and D₂.—Successive sections of stoma bearing six fungi. Those numbered 1 and 2 are nearly empty and a single vesicle (*b*) is forming within. Those numbered 3 and 4 appear separate in D₁; connected in D₂. They have separate germ tubes, (*d* and *e*). Nos. 5 and 6 (in D₁) are younger, and fill in the ends of the stoma. $\times 730$

The fungi which failed to enter were not uniformly distributed on the leaf but occurred in restricted areas. Throughout four or five successive slides all the fungi would have entered; in the next, the majority would still be outside. This may be correlated with the occurrence of patches of open and closed stomata in wheat, as noted by Loftfield (25).

An abundance of spore inoculum was used, and stomata crowded with fungi were of rather frequent occurrence. In Plate 2, A, the highly irregular mass of fungous plasm outside the stoma is drawn at 3 planes in the same section, *a*, *b*, and *c*. At the upper focus, *a*, it appears as two unequal appressoria in contact. At a slightly lower focus, *b*, the two are connected. Still lower, at *c*, the connection is broader. No other fungi or debris were near, and there was no apparent external reason for the irregularity in the shape of this mass. The conformation and the size suggest an incomplete union of two appressoria.

Plate 2, B, represents a similar case. Two successive sections are drawn. In one section, *a*, it appears as two separate bodies; in the other, *b*, the two are connected. The plane of the section is slightly oblique, the bulk of one mass lying in one plane, and the bulk of the other in another. On comparing with Plate 1, A and C, one sees that, in size, it is equivalent to two appressoria.

In Plate 2, C, the two are connected by a narrow bridge, at *a*. In this case entry is in progress, as indicated by a single substomatal vesicle forming at *b*.

In Plate 2, D₁ and D₂, the situation is more complex. Two successive sections are drawn. No fewer than six fungi are present at this stoma. The oldest, numbered 1 and 2, are nearly emptied. No connection between the two was detected, but only one vesicle (D₂, *b*) is forming inside. The appressoria numbered 3, and 4 appeared separate in D₁ and connected in D₂. The empty germ tubes leading into them at D₂, *d* and *e*, allow no question as to the separate origin of the two. Nos. 5 and 6 appear to be younger, and fill in the two ends of the stoma.

In a few cases, as that in Plate 2, E, the two appressoria *a* and *b* remain distinct, effect separate entries, and then the substomatal vesicles *c* and *d* become connected. Another case (pl. 3, A) is an oblique section of both stoma and fungi and is included only because of the surprising similarity of contour and mode of joining, to these features figured in Plate 2, E. These are all drawn to the same scale. On comparing the substomatal vesicles of Plate 2, E, with those in Plate 1, E, one sees that the volume of the united vesicles in Plate 2, E, is at least equal to the total volume of the two separate vesicles in Plate 1, E.

EXPLANATORY LEGEND FOR PLATE 3

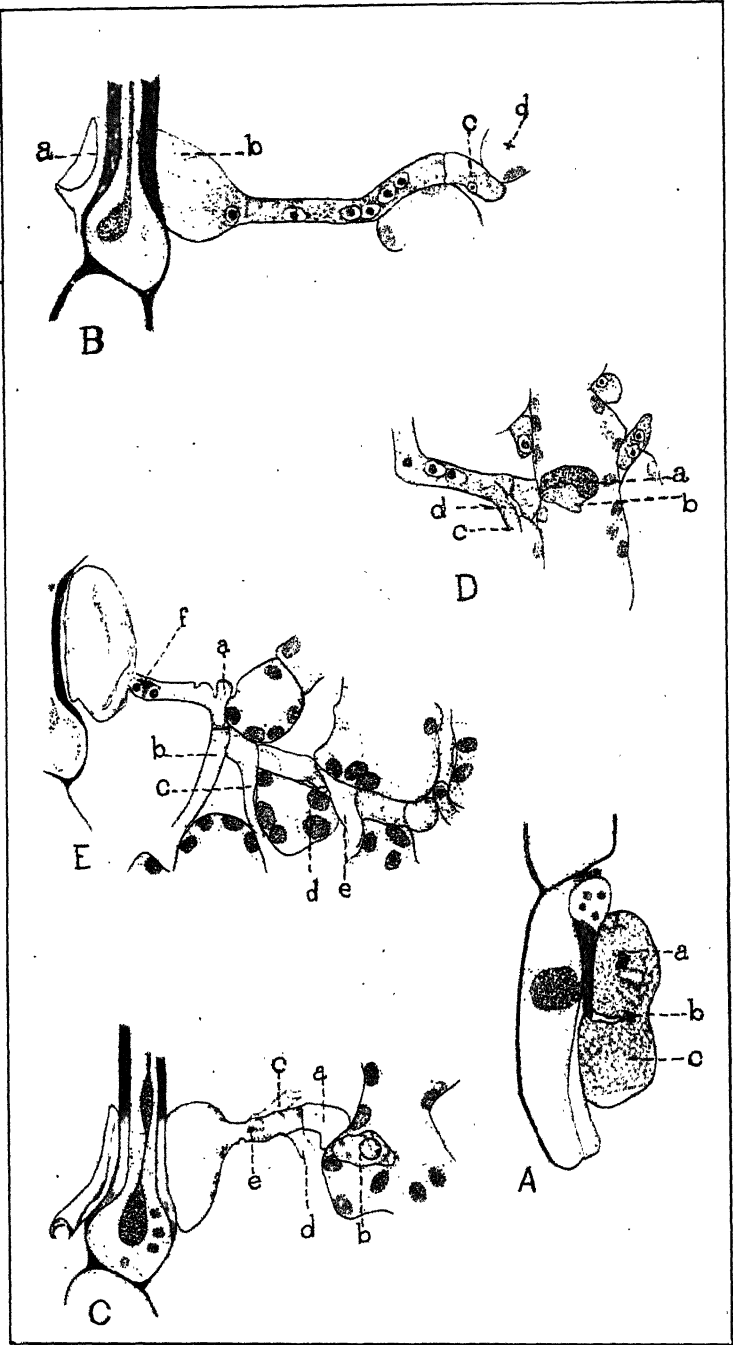
A.—Second day after inoculation. Lateral section of stoma and two substomatal vesicles (*a* and *c*), the latter joined at *b*. × 730

B.—Fungus making first attack. Appressorium at *a*. The substomatal vesicle has formed an infecting hypha with haustorium mother cell *c*. The hypha contains six nuclei. A small haustorium appears in the next section at a point corresponding to *d*. × 730

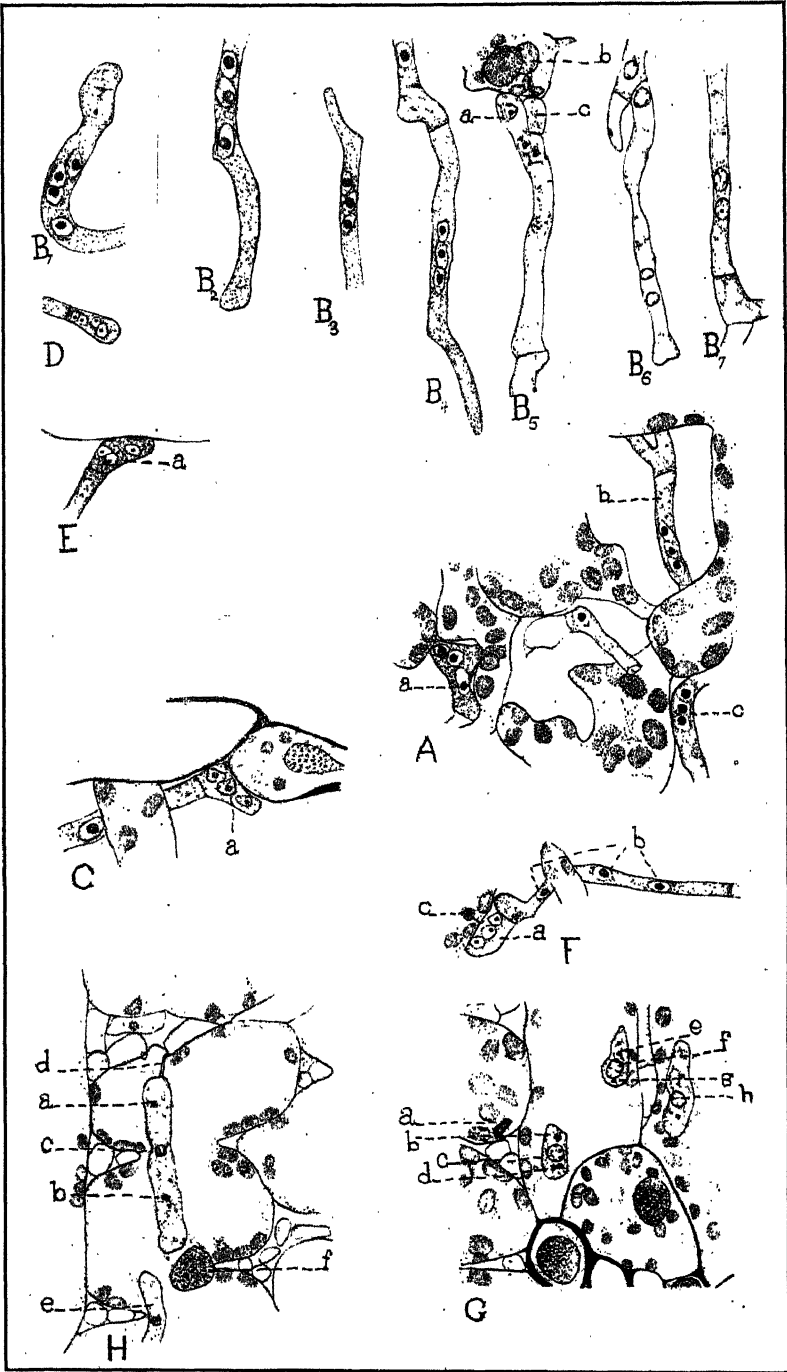
C.—Six-day infection. The first haustorium (*b*) formed from the haustorium mother cell *a*. Host cell apparently is not injured. The infecting hypha gave rise to branches *c* and *d*. One nucleus (*e*) remains in hypha. × 730

D.—Three-day infection. The first infecting hypha formed a haustorium from the mother cell *d*, and then branched at *c*. The haustorium *b* is partly surrounded by the host nucleus *a*. × 730

E.—Six-day infection. The infecting hypha has formed branches at *b*, *c*, *d*, and *e*. Two nuclei (*f*) remain near the base of the vesicle. × 730



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat
(For explanatory legend see p. 204)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat
(For explanatory legend see p. 205)

An attempt has been made to determine whether fusions of germ tubes take place before the formation of appressoria. Spores were germinated on glass slides coated with a thin film of vaseline. The vaseline served the double purpose of stimulating germination and fastening the spores and germ tubes to the slide. Permanent preparations were made. The material was fixed by immersing the slide in fixing fluid, then washed, dehydrated, stained, and mounted in balsam. A rich growth of germ tubes was obtained, but no indication of fusion was noted.

VEGETATIVE GROWTH OF THE FUNGUS

After entry the fungous plasm originally present in the spore is massed in a rounded vesicle just inside the stoma, and this move has been accomplished without food other than that originally stored in the spore. The next step is the formation of the infecting hypha. The hypha grows inward across the air chamber beneath the stoma, taking a course at right angles to the epidermis of the leaf. (Pl. 3, B, C, E.) This is in marked contrast to the behavior of the first hypha in stem rust (2), which skims along the inner surface of the epidermis to the mesophyll cell nearest the end of the stoma.

Cytoplasm and nuclei flow into this infecting hypha (pl. 2, B), leaving the vesicle *b* nearly or quite evacuated. On striking a mesophyll cell (pl. 2, C) a septum may form just back of the tip, giving rise to a short terminal cell, *a*, the haustorium mother cell. The hypha may pass the first cell, however, and not start this process until the second contact. (Pl. 2, B.) In a number of young hyphae, as in Plate 2, B, six nuclei were counted. The nuclear content of the first haustorium mother cell is not easy to determine, because it usually is dense; but, as the vesicle often contains eight and the hypha six nuclei, the haustorium mother cell probably has two.

In Plate 3, B, a dense young haustorium has formed and appears in the next section at a point corresponding to *d*. In the older specimen drawn in Plate 3, C (fixed six days after inoculation), the mother cell *a* is empty and the haustorium *b* has expanded and its nucleus shows the chromatin network and the dense peripheral granule of the typical haustorial nucleus.

In no case observed, up to the sixth day at least, has the host cell suffered visibly from the invasion. It is not plasmolyzed, collapsed, nor even impoverished. The only disturbance noted is in the host nucleus, which may move over to the haustorium. This would appear to be an independent motion of the nucleus, unaccompanied

EXPLANATORY LEGEND FOR PLATE 4

A.—Portion of vegetative mycelium in 6-day infection, showing groups of three nuclei in the hyphae at *a*, *b*, and *c*

B₁ to B₇.—Single hyphae showing nuclear content. B₁ hypha from 5-day infection; cell multinucleate. B₂ and B₃ from 6-day infection, growing tips with three nuclei. B₄, trinucleate cell from 8-day infection. B₅ and B₆, vegetative trinucleate cells from margin of 16-day infection. B₇, pair of nuclei in cell just below epidermis beyond the edge of the uredinium. × 730

C.—Haustorium mother cell (*a*) with three nuclei. × 730

D.—Haustorium mother cell with four nuclei from 5-day infection. × 730

E.—Haustorium mother cell with three nuclei from 12-day infection. × 730

F.—Detail from 5-day infection. Three nuclei in hypha, *b*. Trinucleate mother cell at *a*, young haustorium at *c*. × 730

G.—Nine-day infection. Very young haustorium at *a*. Haustorium at *c*, with one spherical nucleus and granules at *b* and *d*. Haustorium at *f* with granules at *e* and *g*. In the older haustorium (at *h*) the two granules have disappeared. × 730

H.—Large haustorium with one nucleus (*c*) and granules at *a* and *b*. Host nucleus (*f*) midway between haustoria *c* and *e*. × 730

by the other cell contents; at least no massing of cytoplasm and plastids about the haustorium has been noted. In two cases observed, one of which is illustrated in Plate 3, D, the host nucleus *a* is wrapped around the young haustorium *b*, partly inclosing it.

In all cases the infecting hypha gives rise to one or more branches soon after its own advance is checked by the formation of a haustorium. (Pl. 3, C, *c, d*; pl. 3, D, *c*; and pl. 3, E, *a, b, c*.) These branches grow, come in contact with host cells, form haustorium mother cells and haustoria, and in their turn they branch, and repetition of this process soon results in a rich branching system of hyphae. Early in this development the older hyphae are partly or wholly empty, their contents apparently having drained toward the growing tips. This conservation of living material speeds the advance of the fungus.

The nuclear history during this vegetative development has been followed in some detail. Uredinal generations of rusts are considered to be sporophytic, and as a rule are made up of binucleated cells. The physiologic form of leaf rust studied here presents considerable deviation from this rule.

As already noted, the spore has two nuclei, the appressorium four or more, the substomatal vesicle commonly eight, and the infecting hypha, after forming the first haustorium mother cell, often contains six. In the further development, one or two of these nuclei usually are left behind close to the substomatal vesicle (pl. 3, E, *f*; and pl. 3, C, *e*), and the others divide and their progeny become distributed to the branches. Early hyphae of the young fungus have a somewhat irregular nuclear content. Four or even more nuclei in a cell (pl. 4, B₁) are not rare. Soon, however, groups of 3 nuclei (pl. 4, A, *a, b, c*; and pl. 4, B₂ to B₆) become conspicuous.

Hundreds of these groups of three nuclei have been encountered in infections of different ages, grown at different times of the year and on different hosts. It is not a simple matter, however, to determine that vegetative cells are regularly trinucleate. The hyphae are confined to the irregular communicating air spaces of the spongy mesophyll tissue, and it is seldom possible in sectioned material to trace an individual hypha through any great distance. Moreover, the septa delimiting a cell are thin, and in young hyphae with dense cytoplasm they are easily overlooked. When, occasionally, cell limits are clear, especially in older, partly drained hyphae (pl. 4, B, B₅, B₆), it is evident that trinucleate cells are being dealt with. It is not obvious why the number should be three.

This trinucleate condition in a mycelium does not originate in connection with a fusion of two fungi at the beginning of infection. Young fungi occur with characteristic groups of three nuclei, in which it can still be determined with certainty that a single appressorium was present at the point of entry.

Hauatoria form and expand freely. They are regularly uninucleate. In view of the multinuclear mycelium giving rise to them, it was of interest to trace their origin and growth. When the tip of a hypha strikes against a host cell, and its growth in length is forcibly checked for the moment, the changes preparatory to haustorium formation set in. The nuclei divide, one set of daughter nuclei moves out into the thickened tip of the hypha, and a cross wall formed just back of this tip isolates a short terminal cell, the haustorium mother cell. This cell usually flattens out somewhat against

the host-cell wall and often becomes shoe-shaped. (Pl. 4, C, *a*; pl. 3, C, *a*; pl. 3, D, *d*; and pl. 4, E.) It regularly has three nuclei (pl. 4, C, *a*; pl. 4, F, *a*; and pl. 4, E, *a*); rarely two or four (pl. 4, D). These nuclei become markedly reduced in size, as may be seen by comparing them in Plate 4, F, at *a*, with their sister nuclei in the hypha at *b*.

The contents of the haustorium mother cell now pass through the wall into the host cell. First a slender tube forms, extending into the host cell, usually at right angles to the wall at that point. The cytoplasm and the three minute nuclei of the mother cell pass through this tube or "neck" of the haustorium, forming at its inner end a dense globular head within which no details of structure can be seen at first. (Pl. 4, F at *c* and G at *a*.) By absorption of water, it soon expands and the cytoplasm of the haustorium opens into a reticulate structure within which appears a single globular nucleus. (Pl. 3, C, *b*; pl. 4, G, *c, f, h*; and pl. 5, A.) This nucleus contains a rather coarse chromatin net and a single densely stained rounded granule at its periphery.

The question at once arises as to what becomes of the other two nuclei. None remains behind in the mother cell. Do all three fuse to form the single nucleus found in the haustorium, or do two of the three degenerate? A study of half-grown haustoria shows the regular presence of two small dense bodies in the cytoplasm, usually near the nucleus and on opposite sides of it. In Plate 4, G, the haustorial nucleus *c* is attended by small masses at *b* and *d*, and the nucleus at *f* by bodies at *e* and *g*. Sometimes these appear fragmented, as in Plate 5, A, *b, c*. Ordinarily they disappear as the haustorium matures (pl. 5, E, F) but may occasionally still be seen (pl. 4, H, *a, b*). It seems probable, then, that the central nucleus survives and that the other two nuclei degenerate.

H Haustoria extend in all directions within the cell, and it often happens that the neck of the haustorium is removed in sectioning. It is assumed, however, that in life a haustorium continues to be connected by this tube with the mother cell outside. Ordinarily the neck remains slender. (Pl. 4, H, *d*; and pl. 5, E, *a*.) A few cases have been seen, as in Plate 5, F, at *a*, in which the neck shortens and thickens and the host wall near by swells.

* In shape the great majority of the haustoria are cylindrical or worm-shaped. A few (pl. 5, E, *b*) become irregularly branched. They attain large size. In 9-day infections the 10 largest that were measured averaged 39 μ in length, and in 16-day infections they averaged 44 μ .

The nucleus of the host seems to be powerfully attracted by the haustorium. A nucleus is often seen flattened out against it. (Pl. 5, B, *a, b*.) When two haustoria lie a short distance apart (pl. 4, H, *c, e*) the nucleus *f* is often midway between the two. When nearer together (pl. 5, D, *a, b*) the nucleus may be drawn out into a dumb-bell-shaped body (*c*) having contact with both haustoria. Its shape suggests amoeboid motion, but of course in fixed and stained material the evidence is inadequate. An unusual case is seen in Plate 5, C. The host cell is binucleate, and both nuclei (*a, b*) are stretched out against the haustorium, *c*. This contact of nucleus and haustorium does no visible harm to either body; both continue to live and function.

Of rare occurrence is the peculiarly degenerated haustorium figured in Plate 5, G, *a*. It gains significance from the fact that these occur commonly in infections of this rust on less susceptible hosts. Even this dead haustorium is accompanied by the host nucleus, *b*.

REPRODUCTION

Reproductive activity begins early in leaf rust. The first spores are liberated on the eighth or ninth day, or sometimes as early as the seventh. With the onset of reproduction there comes a marked change in nuclear relations. Any section through the margin of a uredinium where the spores are still young and thin-walled (pl. 6, A) shows that spores are uniformly binucleate. A small portion of spore-bearing tissue is drawn on a larger scale in Plate 7, A. Spores (pl. 7, A, *a*, *b*) and stalk cells (*c*, *d*) are binucleate. The reproductive cells beneath are partly drained, the cytoplasm is diminished in amount, and the nuclei are reduced in size and sometimes indistinguishable. Occasionally, however, as in Plate 7, A, *e* and *f*, cells at some depth below the surface are binucleate.

Feeding hyphae at the center (pl. 6, A, B) are too closely interwoven to make it possible to determine cell limits. Moreover, the translocation of food materials from the central mycelium to the reproductive areas at the surface has left these hyphae nearly empty, and their nuclei, when visible at all, are vague.

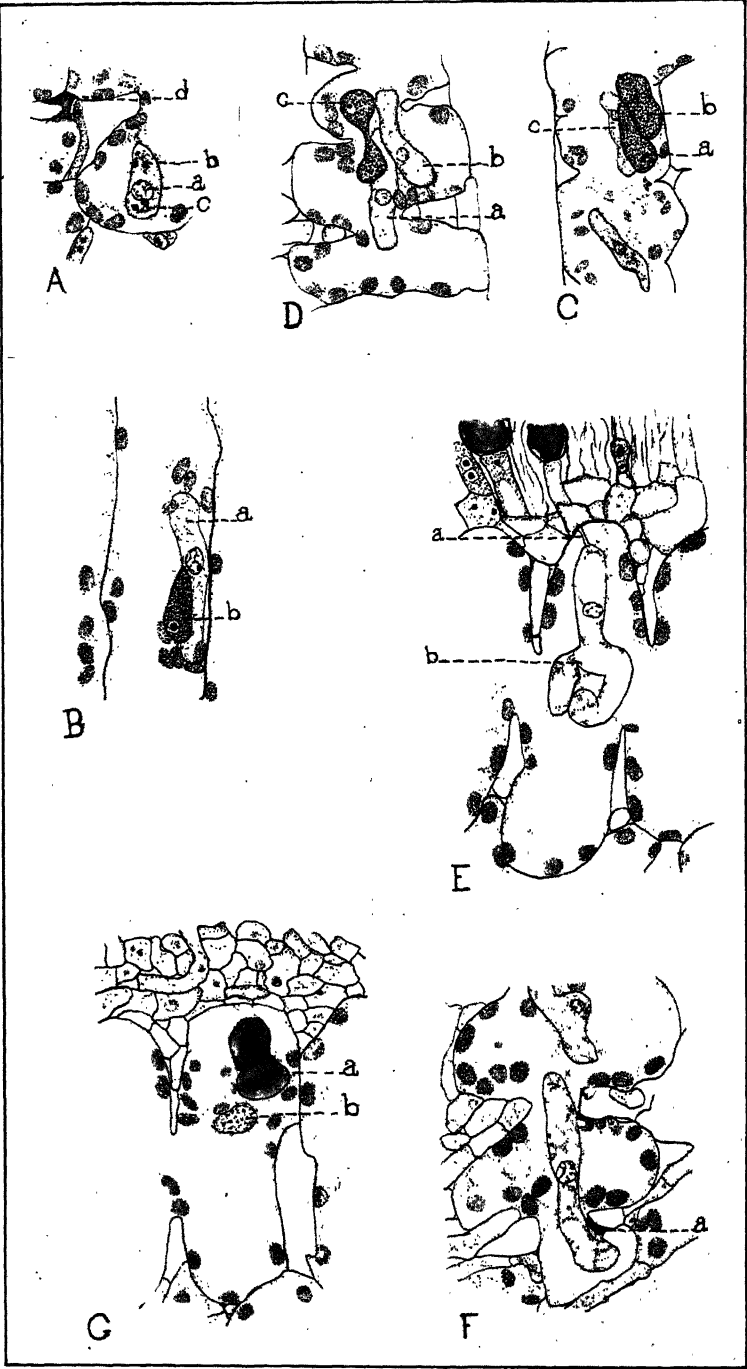
It is in the marginal regions of the infections, if anywhere, that the mode of origin of the binucleate condition is to be determined. Apparently it is in only those cells soon to give rise to spores that this change takes place, for vegetative hyphae of the deeper tissues still show the groups of three nuclei. (Pl. 4, B₅.) Even a week or 10 days after reproductive activities have begun, one still may see them in feeding hyphae in the outskirts of the infection. In B₅ in Plate 4, taken from a 16-day infection, is a young haustorium (*b*) from a mother cell (*c*), and just below it the three nuclei are passing out into the branch at *a*.

But along the line of spore formation just below the epidermis, fungous cells become binucleate. As the uredinium spreads radially, more and more of the subepidermal mycelium changes character, and this takes place considerably in advance of spore formation. Subepidermal binucleate cells may sometimes be found 150 μ beyond the young spores.

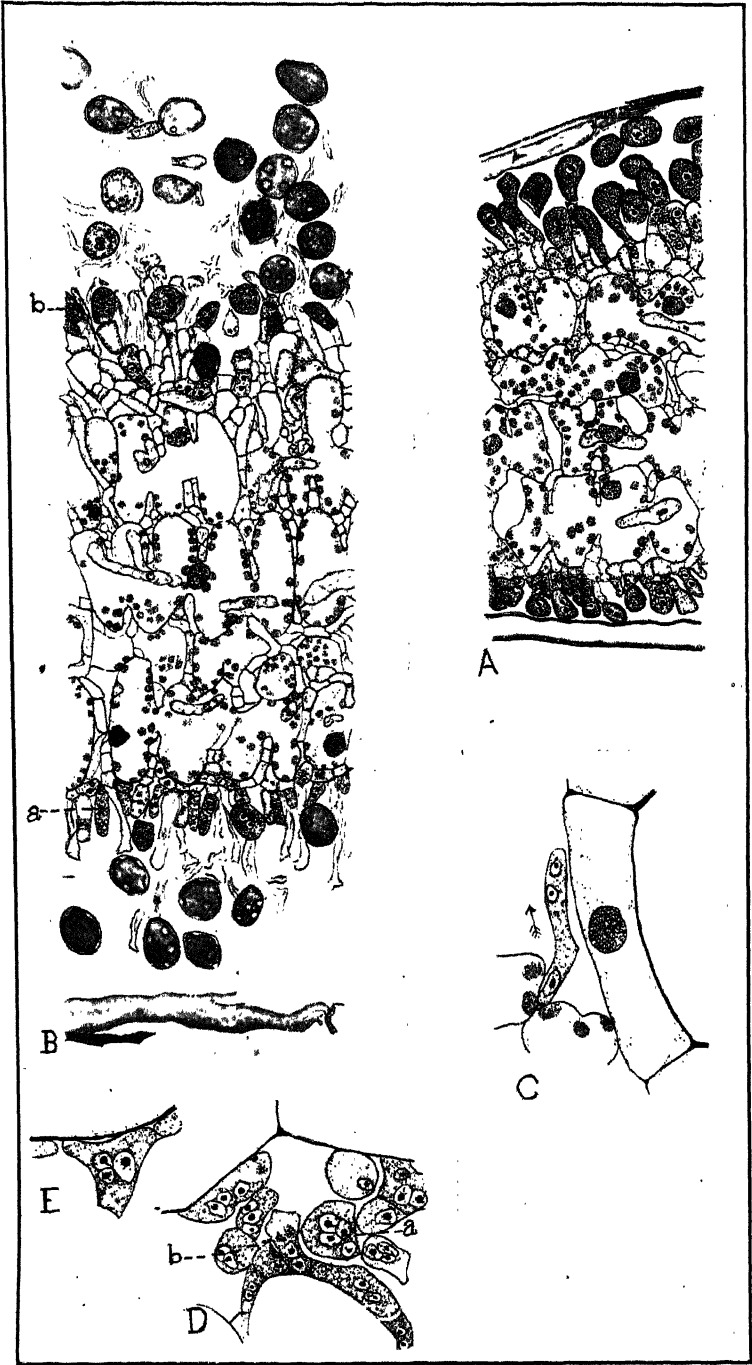
Attempts to discover just how this change takes place have led to rather puzzling results. Theoretically it might happen in any one of several ways. A trinucleate cell might divide into two, leaving one nucleus behind in the penultimate cell and two in the terminal cell. In its further growth the terminal cell would give rise to a series of binucleated cells. This appears to happen in some cases. In Plate

EXPLANATORY LEGEND FOR PLATE 5

- A.—Haustorium with nucleus at *a*, and a group of small granules at *b* and *c*. Host wall swollen and disintegrated at *d*. $\times 730$
 B.—Haustorium (*a*) with host nucleus (*b*) flattened against it. $\times 730$
 C.—Host cell binucleate, and both nuclei (*a* and *b*) are stretched out along the haustorium *c*. $\times 730$
 D.—Dumb-bell-shaped host nucleus *c* making contact with two haustoria, *a* and *b*. $\times 730$
 E.—Branched haustorium (*b*) with long, slender neck (*a*), in 16-day infection. Host cell still vigorous. $\times 730$
 F.—Old haustorium with short, thick neck at *a*, and the near-by host cell wall swollen. $\times 730$
 G.—Detail from 16-day infection. Dead haustorium *a* composed of central core, and a lighter-staining outer layer. Host nucleus at *b*. $\times 730$



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat
(For explanatory legend see p. 208)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat
(For explanatory legend see p. 209)

7, C, there are binucleate cells (*b*, *c*), and back of them, in the same hypha, is a uninucleate cell (*a*).

The binuclear condition also would be established if one nucleus of a trinucleate cell divided, forming a cell with four nuclei, and a cross wall separated it into two cells of two nuclei each. No direct proof of this has been seen. Subepidermal cells containing four nuclei occur. (Pl. 6, D, *a*.) If binucleate cells arose in this manner it would be difficult to prove it after the event, for the result of an ordinary division of a binucleate cell would look the same.

A binucleate cell might arise by the degeneration of one of the three nuclei, by some process similar to that which regularly occurs in the haustorium. This also is not proved, although such cases as the cell in Plate 6, D, *b*, with two normal nuclei and dense granules near by, are suggestive.

Two nuclei of a trinucleate cell might fuse, giving rise to a binucleate cell, but there is no evidence that this occurs. The nuclei of these short cells are usually in contact, and occasionally one sees a large nucleus with two nucleoli, but this in itself is not proof.

In Plate 6, C and E, are two cells each containing one full-grown nucleus and a pair of half-grown nuclei. This condition probably arose by the independent division of one of the nuclei of a binucleate cell. Evidently the simultaneous "conjugate divisions" which maintain the binucleate condition in other rusts are not well established in this readjustment period.

Possibilities of a different type are indicated in the specimens drawn in Plate 7, D and E, one of which (D) was found in a 6-day infection and the other (E) in a 12-day infection. In both of these, two hyphae converged and fused (pl. 7, D and E, at *a*) and an enlarged mass formed at the point of union. In Plate 7, D, the two fusing hyphae (*b* and *c*) can be traced back a considerable distance. One, at least (*b*), is multinucleate. Coming out of the fused mass are one abortive hypha (*d*) and one vigorous hypha which has formed a haustorium mother cell, at *e*. A small haustorium from it appears in the next section, and a branch (*f*) contains a pair of nuclei. Plate 7, E, is similar in essentials. The mycelium near by is mainly binucleate.

This process suggests at once the fusions of two separate fungi noted at the stoma at the beginning of infection. The question arises as to the parentage of the fusing hyphae here. When adjoining stomata are entered by individual sporelings, their resultant mycelia become intimately intermingled. Under such circumstances adjoining hyphae might come from different parents.

In neither of these cases is the evidence absolute. In the section, a portion of which is figured in Plate 7, D, there are several young infections slightly overlapping. The hyphae that fuse can be traced back toward the point of entry. The point *g* is only 50 μ away from the original infecting hypha at the stoma, and the other

EXPLANATORY LEGEND FOR PLATE 6

A.—Marginal portion of a uredinium in a 9-day infection. Host tissues vigorous. Young spores and stalk cells binucleate. $\times 333$

B.—Portion of section through 16-day infection. Fungus not massive, but spore production heavy at both surfaces. Host tissues living and not seriously impoverished. $\times 333$

C.—Fungous cell just below epidermis, 200 μ beyond margin of uredinium. Contains a pair of young nuclei at the tip and one large nucleus at the base. $\times 730$

D.—Subepidermal mycelium in the region of transition to binucleate condition, containing two to four nuclei per cell. Four nuclei in cell at *a*. At *b*, cell with two nuclei and two dense granules. $\times 730$

E.—Cell just below epidermis, with pair of small daughter nuclei and one full-grown nucleus. $\times 730$

hypha (c) came from the same direction. The probability is that in this case the fusing hyphae are rather closely related. In an older infection this would be still harder to determine, for the early history of the fungus is more or less obliterated by the later growth. In the older infection from which Plate 7, E, was drawn, only one discolored stoma was noted (indicative of the point of entry of the fungus), but, as part of the epidermis over the open uredinium was missing, it is possible that more than one mycelium was present.

How frequently and under what conditions this type of fusion occurs is not known. Both of these cases were found in the relatively free space of a substomatal cavity. In almost any other location they would pass unnoticed. Even here they would have been overlooked after other hyphae had closed in around them.

Taking place as it does in tissues about to form spores, this fusion also is vaguely suggestive of the fusions which occur at the base of the aecium in other rusts. Nothing is known of the nuclear phenomena here, nor of the later development from the fusion, nor what share, if any, the fusion has in the establishment of the binucleate spore-bearing tissue.

By whatever process or processes the shift to the binucleate condition takes place, it works efficiently. The mother cell of the spore (pl. 7, A, g) is regularly binucleate, and upon its division the spore and the stalk cell each receive 2 nuclei. (Pl. 7, A, a, b, c, d.)

As the spores mature they round up, their wall thickens, and their stalks elongate. When the stain is favorable, a number of scattered germ-pores in the spore wall can be seen. (Pl. 6, B.) The spores are set free by the rupture of the epidermis. The spore stalks wither and new spores push out between them. (Pl. 6, B, a, b.) This process continues as long as food is available.

In spite of the congeniality of parasite and host, the leaf-rust mycelium in seedling leaves does not attain a very massive growth nor any great diameter. The limited spread of *Puccinia triticina* within the leaf may be explained by the weak development of "runners" or "stolons." Even in 16-day infections the marginal growth of the fungus is composed of ordinary feeding hyphae with haustoria at frequent intervals. The long, straight, rapidly growing, sparsely septate and sparsely branched stolons, so common in stem-rust infections from the sixth day on, are rarely encountered here. Secondary uredinia do occur, however, under favorable conditions. Plate 8, A, is reproduced from a photograph of a 25-day culture on Trumbull, a very susceptible variety of wheat. The circle of secondary uredinia is separated by a narrow clear space from the primary. No second circle of uredinia has been noted. A comparison of this with the corresponding development of stem rust under favorable conditions shows a marked contrast. In Plate 8, B, is shown, at

EXPLANATORY LEGEND FOR PLATE 7

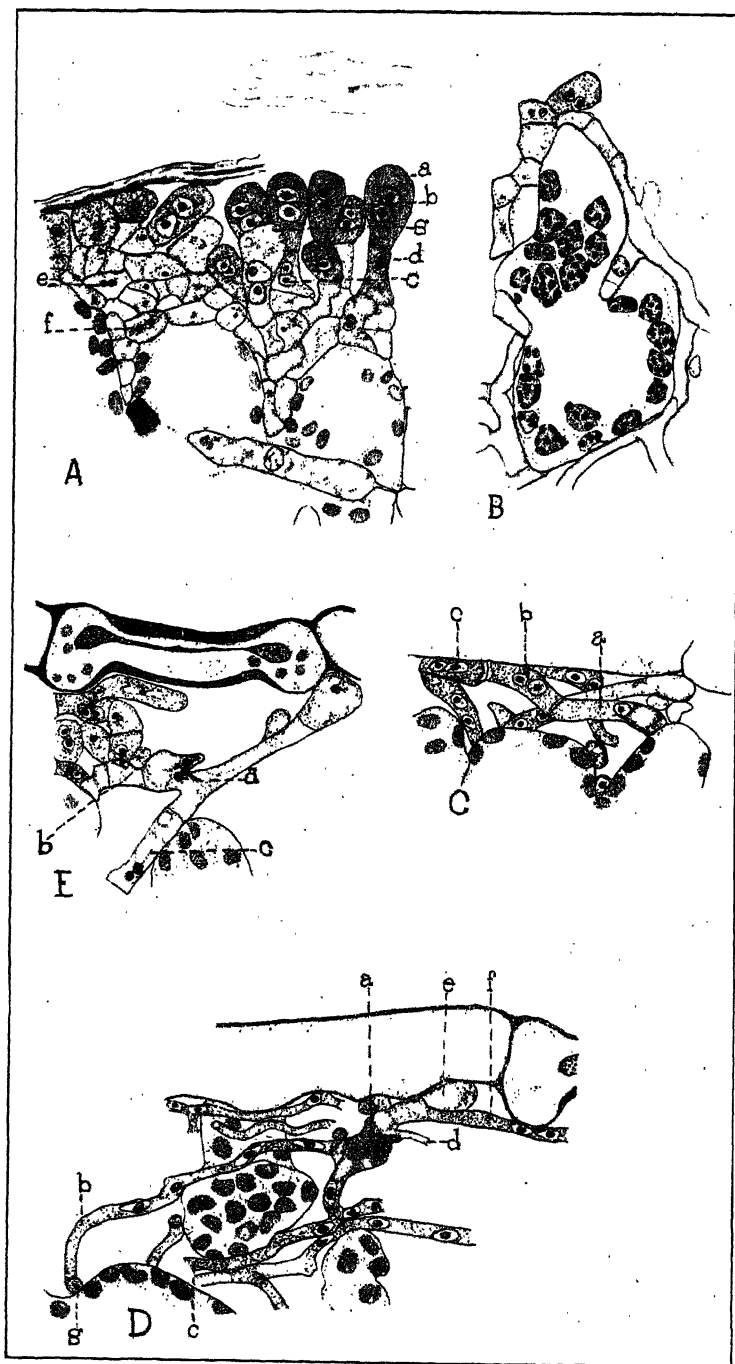
A.—Margin of uredinium from 9-day infection fixed at 4 p. m. Spores (a, b), stalk cells (c, d), and deeper lying cells (e, f) binucleate. Starch content of host tissue moderate. $\times 730$

B.—Cell just below the uredinium of a 10-day infection on a weakened leaf. Plastids with maximum starch content. $\times 730$

C.—Subepidermal mycelium near margin of uredinium. Hypha with uninucleate cell at a, and two binucleate cells at b and c. $\times 730$

D.—Portion of subepidermal mycelium from 6-day infection near stoma of entrance. Cells chiefly trinucleate. Hyphae b and c meet and join at a. Proceeding from a are a vegetative hypha with haustorium mother cell at e and branch at f, and an aborted hypha at d. $\times 730$

E.—Mycelium in substomatal cavity at margin of 12-day uredinium. Cells chiefly binucleate. Cells at b and c fuse at a. From the point of union proceed an aborted tip and a vigorous vegetative hypha. $\times 730$



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 210)

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EXPLANATORY LEGEND FOR PLATE 7

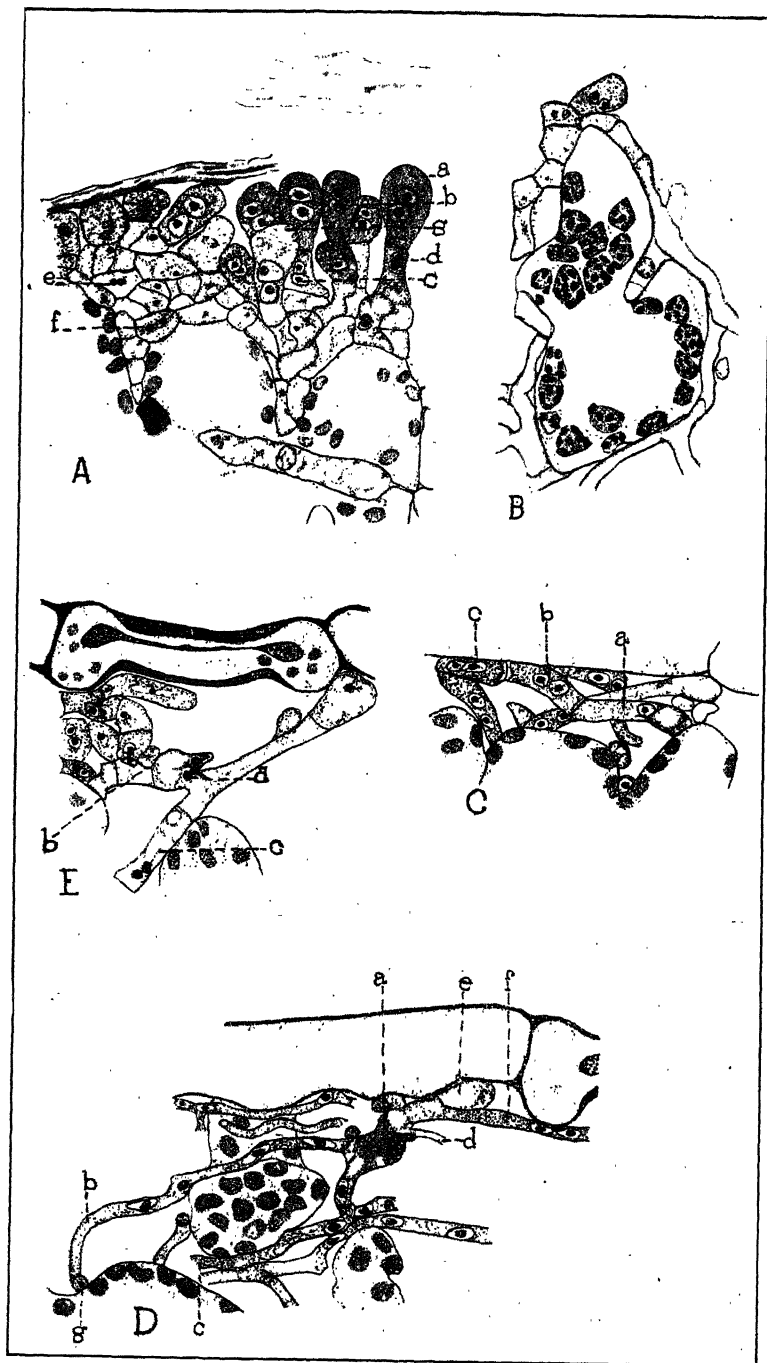
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B.—Cell just below the uredinium of a 10-day infection on a weakened leaf. Plastids with maximum starch content. $\times 730$

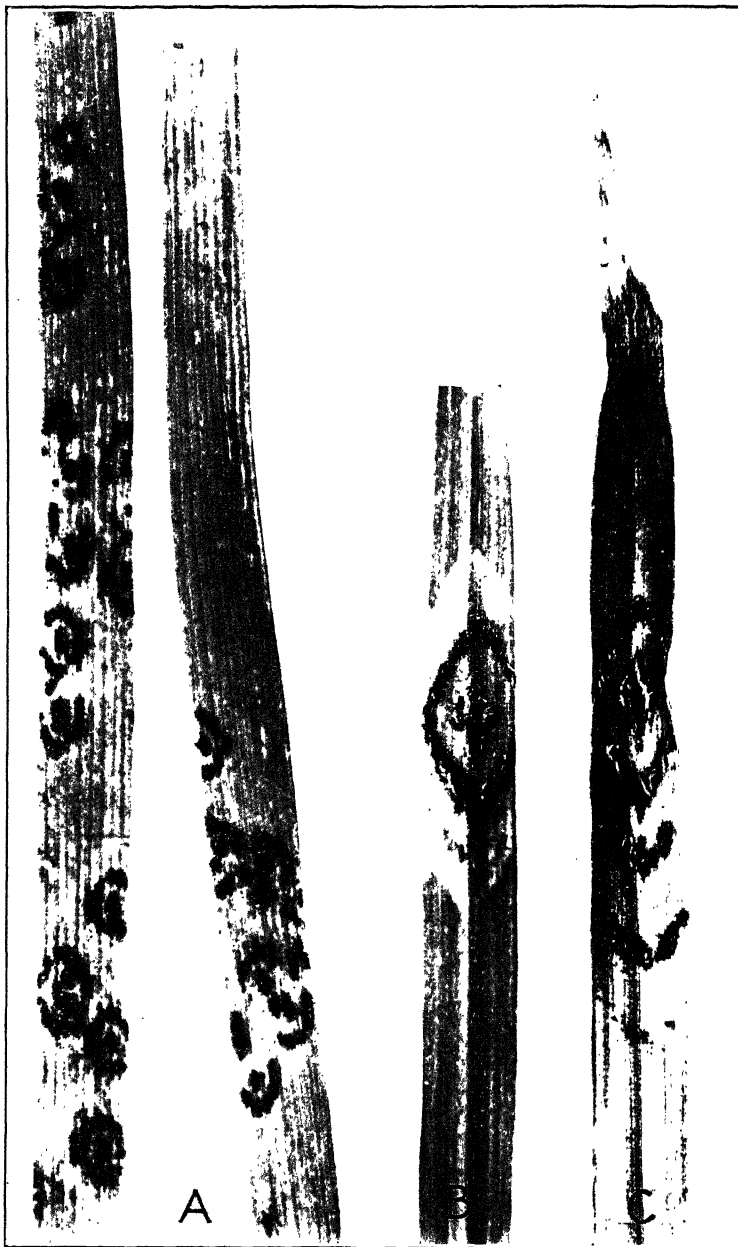
C.—Subepidermal mycelium near margin of uredinium. Hypha with uninucleate cell at a, and two binucleate cells at b and c. $\times 730$

D.—Portion of subepidermal mycelium from 6-day infection near stoma of entrance. Cells chiefly trinucleate. Hyphae b and c meet and join at a. Proceeding from a are a vegetative hypha with haustorium mother cell at e and branch at f, and an aborted hypha at d. $\times 730$

E.—Mycelium in substomatal cavity at margin of 12-day uredinium. Cells chiefly binucleate. Cells at b and c fuse at a. From the point of union proceed an aborted tip and a vigorous vegetative hypha. $\times 730$



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat
(For explanatory legend see p. 210)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

A.—*Puccinia triticina* physiologic form 11 on Trumbull wheat grown in greenhouse. Rust 25 days old. Scant development of secondary sori. $\times 3$.

B.—A single-spore, 23-day culture of *Puccinia graminis tritici* physiologic form 3 on Little Club wheat, showing secondary uredinia. $\times 3$.

C.—Same culture as in B, at 39 days of age, showing further development of secondary uredinia. $\times 3$.

the same magnification, a single-spore infection of stem rust (*Puccinia graminis tritici* physiologic form 3) 23 days old on Little Club. Plate 8, C, shows the same infection at 39 days. Three or four concentric lines of uredinia have formed at broad uniform intervals. The total spread of the fungus is six or eight times that of leaf rust.

RELATION TO THE HOST

The host tissues in and about the infection show remarkably little disturbance in either cell walls or cell contents. Except for the modification of guard-cell walls at the point of entry of the fungus, there is very little effect on the cell walls of Little Club. As far as has been observed, it seems to make very little difference whether the host is young or old, or whether the fungus is grown under favorable or unfavorable conditions; the walls of the host plant rarely are affected. Once in a while a limited spot is swollen (pl. 5, A, *d*), but this is exceptional.

Another phase of the interaction of host and parasite is the heightened osmotic pressure of infected tissue. In all but the youngest infections a fixing fluid which will fix the fungus and the infected host tissues well, often will shrink the healthy tissues of the leaf. It is not clear whether this increased turgor is due directly to some substance secreted by the fungus into the host cell or to the heightened or altered activities of the cell in response to the fungus or to a combination of the two.

The infected tissues live on. Sometimes there is not a single dead cell in 9-day and 12-day infections, and even in 16-day infections there is not more than 1 per cent of dead cells. In former stem-rust studies it was noted that even in fairly congenial relationships the first host cell attacked by the young fungus often rapidly collapsed and died. Here, on the contrary, the cell containing the first haustorium is alive and apparently functioning normally on the fifth or sixth day of the infection. Later than this it is not always possible to identify the first cell, for the early history often is obscured by the massing of the fungus below the epidermis for reproduction. The one or two dead cells in old infections are at the center of the infection and just below the epidermis, however, and may well be the first cells invaded by the fungus.

A closer study of the conditions in infected host cells shows even more clearly how slight are the visible alterations in cell activities. The size of plastids, except when they are gorged with starch, is an index of the vigor of the cell, for in impoverished tissues plastids become reduced and disappear.

Plastids were measured in infections of different ages (Table 1), at the center and the margin of the infection, 50 μ beyond the outermost hypha, and at a distance far enough away to be beyond any influence of the fungus. Each figure in the table represents the average of 20 plastids, 5 from each of 4 infections. Only plastids in the first layer of cells below the epidermis were measured.

The data in Table 1 indicate that there is very little impoverishment of the host tissues in and about the infections. In the 5-day material the normal plastid is $4.4 \times 2.3 \mu$, and at the center of the infection it is $3.9 \times 2.5 \mu$. In the 16-day material the normal plastid is $4 \times 2.2 \mu$, and at the center it is $3.5 \times 2.3 \mu$. Allowing for the normal

decrease of plastid size in the older leaves, the reduction in size in the older infections is relatively little greater, if any, than in the younger ones. There is a scarcely perceptible impoverishment of tissues beyond the fungus.

TABLE 1.—*The size of plastids in and around infections of Puccinia tritici physiologic form 11 of different ages on Little Club wheat. (Each figure represents the average size in μ of 20 plastids, 5 from each of 4 infections)*

Age of infection	Size of plastids			
	In center of infection	In margin of infection	50 μ beyond margin	Normal
5 days.....	3.9 \times 2.5	3.9 \times 2.4	4.4 \times 2.3	4.4 \times 2.3
9 days.....	4.0 \times 2.5	3.9 \times 2.3	4.0 \times 2.2	4.2 \times 2.2
12 days.....	3.7 \times 2.5	3.6 \times 2.5	4.1 \times 2.7	4.2 \times 2.5
16 days.....	3.5 \times 2.3	3.6 \times 2.1	3.8 \times 2.2	4.0 \times 2.2

The starch content of these plastids is of interest. A comparison was made of infections of different ages in two sets of seedlings grown under different conditions. (Table 2.) The first set was grown in the field in the spring of 1924 in clear, dry weather; the second, third, and fourth leaves were inoculated; the infected leaves were turning yellow at the end of the series. The second set was grown a year later in a wet season with intermittent dark days; the fourth to the sixth leaves were inoculated; the infected plants grew vigorously, kept their color, and a heavy crop of uredinia developed. In both sets, material was fixed at intervals during the growth of the fungi, and slides were prepared. The range of starch content in each lot was estimated. In grading it, S_0 signifies no visible starch, S_3 the maximum, and S_1 and S_2 intermediate quantities. Each figure in the table is the result of observations on three to six infections.

The contrast between the two sets is marked. (Table 2.) The second set of plants grew well in spite of the heavy infection, and the starch content of the plastids within the infected areas was nearly equal to that beyond them. With one exception, the leaves were fixed in the morning, when normally there is little starch left in the leaf. Where there was a difference between the starch content of the healthy and the infected tissues, the infected tissue contained the most, but the difference was slight. The 9-day material was fixed in the afternoon. The healthy tissue was slightly higher in starch; the infected tissue contained about the same amount of starch as when fixed in the morning.

On the contrary, there was a markedly greater amount of starch in the infected tissue, in the 1924 series. (Table 2, set 1, also fixed in the morning.) The starch content increased up to the tenth day, when limited areas in the center of the infection showed the maximum and the plastids were gorged and swollen with starch. (Pl. 7, B.) The contrast between this and the condition on the ninth day in the 1925 set (pl. 7, A) is striking. The leaf fixed on the fourteenth day was beginning to turn yellow, and plastids outside of the infections were smaller. At the center of the infection the starch was about gone, but near the margin it graded S_2 . The situation was substantially the same on the seventeenth day.

TABLE 2.—Summary of starch content of plastids in two sets of infections of *Puccinia triticina* physiologic form 11 on Little Club grown in the field, graded from *S*₀ (no visible starch) to *S*₃ (the maximum)

Set 1 Sown Apr. 7, 1924; inoculated Apr. 30, 1924 (dry season)			Set 2 Sown Mar. 5, 1925; inoculated May 11, 1925 (wet season)		
Age of infection	Starch in plastids in infected tissue	Starch in plastids in uninfected tissue	Age of infection	Starch in plastids in infected tissue	Starch in plastids in uninfected tissue
6 days...	<i>S</i> ₁ -to <i>S</i> ₂ -----	<i>S</i> ₂ to <i>S</i> ₁ -----	5 days...	<i>S</i> ₀₊ to <i>S</i> ₂ -----	<i>S</i> ₁ to <i>S</i> ₁₊ ..
8 days...	<i>S</i> ₁ -to <i>S</i> ₁ -----	<i>S</i> ₀₊ to <i>S</i> ₁ -----	9 days at		
10 days...	<i>S</i> ₂ to <i>S</i> ₃ . Plastids enlarged.	<i>S</i> ₀₊ to <i>S</i> ₁ -----	4 p. m...	<i>S</i> ₀₊ to <i>S</i> ₂ -----	<i>S</i> ₀₋ to <i>S</i> ₂ ...
14 days...	<i>S</i> ₀ at center of infection to <i>S</i> ₂ at its margin.	<i>S</i> ₀₊ to <i>S</i> ₁ .. Leaf turning yellow. Plastids reduced in size.	12 days...	<i>S</i> ₁ -to <i>S</i> ₂ -----	<i>S</i> ₁ -to <i>S</i> ₁ ..
17 days...	<i>S</i> ₀₊ at center to <i>S</i> ₂ at the margin.	<i>S</i> ₀ to <i>S</i> ₁ -----	16 days...	<i>S</i> ₀ to <i>S</i> ₁ -----	<i>S</i> ₀ to <i>S</i> ₁ ..

Further information concerning conditions in infections is to be gained by study of the nuclei. Nuclei were measured at the center of infections of different ages and in tissues of the same leaf far enough removed from the fungus not to be influenced by it. The data are given in Table 3. Each result represents the average of 20 nuclei, 5 from each of 4 infections. The normal nuclei vary a little in size. The writer has observed that the nuclei of the cells which contain haustoria are always a little larger than the corresponding nuclei of healthy tissues, and different in shape. The maximum occurs in 12-day infections where the normal nucleus is 8.5×6.2 μ, and the nucleus in diseased tissue is 10.6×6.3 μ. The increase in volume is not so great as the dimensions indicate, however. There is a tendency for the nucleus to stretch out toward or along a haustorium. The diameter of the body of the nucleus remains unchanged, and the long tapering point at one end represents a relatively slight increase in volume. Not all nuclei undergo this elongation, but, taking them as they come, they decidedly affect the average length of the nuclei. This is in marked contrast to a number of cases in stem rust of wheat, in which nuclei increased in volume several fold and then collapsed and died.

TABLE 3.—Size (in μ) of living nuclei in infection and normal tissue of Little Club seedlings infected with *Puccinia triticina* physiologic form 11. (Each figure represents the average of 20 nuclei, 5 from each of 4 infections)

Age of infection	Size of nuclei	
	At center of infection	In normal tissue
5 days-----	8.8×7.0	8.2×7.1
9 days-----	10.1×6.3	8.6×6.5
12 days-----	10.6×6.3	8.5×6.2
16 days-----	9.1×7.4	8.7×7.4
Average-----	9.6×6.7	8.5×6.8

Relatively few nuclei die, and the number seems to vary somewhat with the vigor of the rust-infected leaf. Counts were made in several infections of 5, 9, 12, and 16 day material. A nucleus was con-

sidered dead when it had shrunk somewhat and stained a homogeneous red in which no chromatin net was discernible. Fewer than 10 per cent of the nuclei had died, even in the oldest infection studied. (Table 4.) In the exceptionally vigorous tissues of the 12-day infections less than 2 per cent of the nuclei died.

TABLE 4.—Percentage of dead nuclei in infections of *Puccinia triticina* physiologic form 11 on Little Club seedlings. (Several infections were included in the count for each age)

Age of infection	Number counted	Number dead	Percentage dead
5 days.....	150	3	2.0
9 days.....	530	39	7.3
12 days.....	698	13	1.9
16 days.....	1,056	99	9.4

INFECTIONS ON OLDER HOST PLANTS

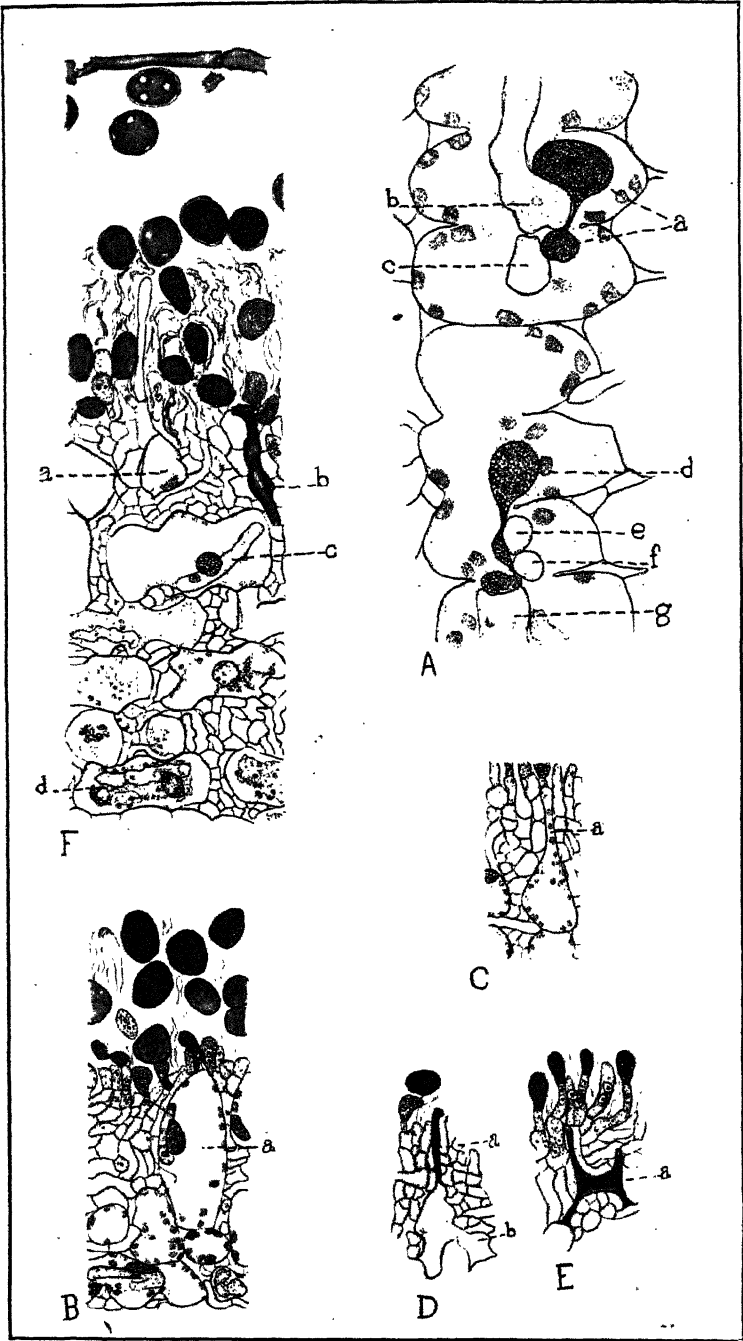
A brief comparative study was made of this strain of leaf rust on older plants of Little Club. The seedlings were grown in the field and inoculated artificially. In their later growth the rust spread naturally, infecting the new leaves as they grew. All stages of the development of the rust were present, but the exact age of any particular infection was not known. Material was fixed at three stages—(1) when the culms began to push up; (2) when they were half grown; and (3) the uppermost leaf, when the heads were 3 or 4 inches out of the sheath.

A cytological study of these infections showed only minor differences. The later leaves had more substance than the seedling leaves, and the fungus made a heavier, more massive growth on them. The sori were longer, and the spore output of each sorus was greater than on the seedlings.

In the details of the relations of rust and host there were few differences. Here, as before, there was a luxuriant development of haustoria, but the average length of the largest ones seen was 44 μ , about the same as in the seedlings. Here, too, the invaded host cells maintained their vigor and appeared to function almost normally. The starch content of the plastids within the infections was equal to or slightly greater than that in healthy tissue. The host nucleus was in contact with the haustorium. It usually was spherical or nearly so, but may have been drawn out irregularly, especially if two or more haustoria occurred in one cell. In Plate 9, A, are two unusually distorted nuclei. The nucleus *a* came in contact with the haustorium *b*, then extended into a lobe almost disconnected with the main body of the nucleus, which forms contact with a second haustorium (*c*). In an adjoining cell (pl. 9, A) the nucleus *d* is

EXPLANATORY LEGEND FOR PLATE 9

- A.—Detail of mycelium. Lobed host nucleus *a* in contact with haustoria *b* and *c*. The much-elongated host nucleus *d* has formed contacts with haustoria at *e*, *f*, and *g*. $\times 730$
 B to E.—Stages in the crushing of subepidermal mesophyll below uredinia.
 B.—Hypodermal cell *a* somewhat narrowed, due to lateral pressure induced by the mycelium. $\times 333$
 C.—Increasing pressure has reduced outer half of mesophyll cell *a* to a mere slit. $\times 333$
 D.—Mesophyll cell *b* dead. No cell lumen left in outer half, *a*. $\times 333$
 E.—Mesophyll cell *a* completely crushed. $\times 333$
 F.—Strip part way through leaf bearing old uredinium. Partly crushed host cell at *a*. Completely killed host cell at *b*. Host cell at *c* still living. Deeper-lying host cells, as at *d*, are not crushed but are plasmolyzed. $\times 333$



Puccinia triticina physiologic form 11 on heading plants of Little Club wheat
(For explanatory legend see p. 214)

tion occurs either by the migration of a nucleus from one cell to another (4, 5, 28, 24) or by the resorption of the walls between two adjoining uninucleate cells. (6, 7, 5, 28, 16, 33, 24.) The layer of binucleate or sporophytic cells so initiated gives rise by conjugate divisions to binucleate aeciospores. The sporophyte continues on through the uredinal generations. Only in the young teliospores do the two nuclei fuse (32). Upon the germination of the teliospore the fusion nucleus divides twice and there is evidence (32, 19, 4, 27) that these are reduction divisions. The sporidia borne on the promycelium initiate the gametophyte once more.

Numerous deviations from this regular cycle are recorded in the literature of rust cytology, and to these can be added the minor aberrations found in *Puccinia triticina* physiologic form 11.

Puccinia triticina can continue to live for an extended period by means of an unbroken line of uredinal generations. Indeed, the probability is that it must do so in America, as the aecial host is rarely present. In answer to an inquiry on this point, E. B. Mains states:

In regard to your question as to the per cent of cases in which the American leaf rust (of wheat) completes its life cycle, I believe that this very seldom occurs. Our work indicates that the only susceptible species of *Thalictrum* are one or two European species, especially *Thalictrum flavum*.

Teliospores form, to be sure, and germinate (20), but in the absence of the aecial host this is a blind alley in the life cycle, and the only effective continuance is by means of urediniospores.

This long-continued uredinal series takes place without apparent diminution in the vigor of the fungus. Cytological study of this physiologic form of the rust, however, reveals a number of irregularities which might be variously interpreted as signs of oncoming degeneration or as sporadic ill-defined ways of compensating for the missing sexual part of the cycle. Briefly, these are (1) the occasional fusion of two appressoria or of two substomatal vesicles; (2) regularly multinucleate cells in the first hyphae, followed by (3) the conspicuous groups of three nuclei and trinucleate cells throughout the vegetative development; (4) regularly uninucleate haustoria from trinucleate haustorium mother cells; (5) occasional fusions of hyphae where spores are soon to be formed; and (6) a complete return to the binucleate condition in the spore-bearing tissue, which then produces normal binucleate spores.

What weight should be given to these variations is a question. No comparative study has been made of this rust passing normally through its complete life cycle. This makes difficult an evaluation of the irregularities found here. A comparison of them with the unusual nuclear behavior found elsewhere in the rusts may be of interest.

Multinucleate cells in the first hyphae of the uredinal mycelium of *Puccinia triticina* were observed by Pole-Evans (15) in 1907. He observed them at a similar stage in *P. phleipratensis*, *P. glumarum*, *P. dispersa*, *P. simplex*, *P. coronifera*, and *P. sorghi*. He seems to regard them as cases of delayed septation. Ward (34) figures two to four nuclei in cells of the young uredo mycelium of *P. dispersa*.

Multinucleate cells frequently have been found at other points in the life cycle. Olive (28, 29, 30) finds that a large proportion of 50 species studied showed multinucleate cells at the base of the aecium

associated with the process of fusion. Fromme (17) finds them at the base of the aecium in *Melampsora lini*.

Lindfors (24) finds that the uninucleate mycelium of the primary uredo of *Trachyspora alchemillae*, which spreads throughout the host plant, may become multinucleate in the cramped quarters of the embryonic tissues of the host, but becomes uninucleate again as those tissues expand. He believes that this should be considered as essentially uninucleate. In *Puccinia glumarum* the uredo mycelium is multinucleate, but it becomes binucleate in reproductive areas. He says (24, p. 28, 30):

Nun geschieht auch die Begrenzung der Kernanzahl auf je zwei in jeder Zelle [of the spore-bearing tissue] teils durch Wandbildung, teils wahrscheinlich auch durch Degeneration eines Teils der Kerne. * * * das Myzel, trotz Vorkommens vieler Kerne in einem Zellenleib während der vegetativen Entwicklung, dennoch im Prinzip als zweikernig zu betrachten ist, was aus dem Verhalten der Kerne bei Bildung und Keimung der Uredosporen hervorgeht.

In the cases just cited the nuclei are numerous and indefinite in number and throw little light on the groups of three nuclei found in the later vegetative development of *Puccinia triticina* physiologic form 11.

Trinucleate cells have been figured by a number of students of rust cytology, but are quite uniformly sporadic cases of triple fusion. At the point of transition from the uninucleate to the binucleate condition (at the base of the aecium in the long-cycle rusts, and in the primary uredo or the telium in those of abbreviated cycles), in a few cases three nuclei become associated instead of two. Spores arising from these trinucleate basal cells also are trinucleate. The fate of these spores is unknown. This has been reported by Blackman in 1904 (4); Blackman and Fraser, 1906 (5); Olive, 1908 (28); Dittschlag, 1910 (10); Hoffmann, 1911 (18); Fromme, 1912 and 1914 (16, 17); Kunkel, 1914 (22); and Lindfors, 1924 (24). These, too, are of little aid in explaining the present case, as the uredinio-spores giving rise to the mycelium of *Puccinia triticina* are binucleate.

It may well be that one should extend Lindfors' recommendation to cover this case and consider it equivalent to the binucleate condition because it begins and ends binucleate. Even so, it remains unexplained why the number of nuclei here should be predominantly and almost uniformly three through all but the youngest vegetative development.

The production of regularly uninucleate haustoria from trinucleate haustorium mother cells is not far out of line with other rusts, but thus far the writer has found no exact parallel in the literature.

Sappin-Trouffy (32), in extensive studies of representative genera throughout the Uredineae, found regularly uninucleate haustoria produced by mycelium composed of uninucleate cells, and either uninucleate or binucleate haustoria (or both) produced by mycelium composed of binucleate cells.

Holden and Harper, in 1903 (19), found that the haustoria of *Coleosporium sonchi-arvensis* were regularly binucleate, like the mycelium which bore them.

Ward, in 1903 (34), studying the mycelium of *Uredo dispersa*, drew one to six nuclei in vegetative cells, two or three nuclei in haustorium mother cells, and one or two (usually one) in haustoria.

Blackman, in 1904 (4), working on *Phragmidium violaceum* found paired nuclei in haustoria of binucleate mycelium. Blackman and

Fraser, in 1906 (5), found two nuclei in haustoria in the binucleate mycelium near the teliospores of *Uromyces ficariae*.

Pole-Evans, in 1907 (15), described the uredo mycelium of *Puccinia phleipratensis* as having two or more nuclei in the haustorium mother cell and two in the haustorium. The multinucleate cells of *P. glumarum* produce haustoria with as many as five nuclei. In the uredinal mycelium of *P. dispersa* a uninucleate haustorium is figured.

Dodge, 1918 (11), speaking of the telial mycelium of *Gymnosporangium macropus*, said: "The haustoria take a very delicate stain, showing the single nucleus in each, or two nuclei when two are present."

Thurston, in 1923 (33), studying *Gymnosporangium bermudianum*, found uninucleate haustoria from binucleate mycelium.

The present writer, in 1923 (3), found one nucleus in the haustoria of *Puccinia graminis tritici*.

This summary is not complete, but as far as it goes it would indicate that the haustoria of different rusts have from one to five nuclei; that the number is sometimes variable, even on the same mycelium; and that the number of nuclei in the haustoria is either the same as that of the mycelium bearing them, or somewhat less. The situation in *Puccinia triticea* physiologic form 11 is in line with this, in that the number in the haustoria is reduced but appears to be more definite and limited than in the similar cases cited.

The significance of the joining of the rust sporelings at the stoma is not known. Not all of the literature has been covered, but no case like it has been noted. The frequency of its occurrence naturally depends, for one thing, on the number of appressoria present. An abundance of spores was used in the inoculation of this material, but some stomata were free, many carried one sporeling, and comparatively few carried more. Even when appressorium are grouped at a stoma they may remain separate and enter and develop independently of each other. Of all the rust sporelings studied in this material, about 4 per cent showed fusion.

Not enough is known of the details of the fusions at the stoma and when spore bearing is imminent, to determine whether they have sexual significance or are merely anastomoses of a vegetative nature. Pairing of nuclei or nuclear fusion has not been proved.

Even a brief survey of rust cytology suffices to impress one with the plasticity of the nuclear history as shown in rusts with altered and abbreviated life cycles. Blackman (4), Dittschlag (10), Fromme (16), Lindfors (24), and others thought that even the regular process of fusion at the base of the aecium was a substitute for an earlier form of reproduction in which the pycniospores functioned as male cells, an opinion that is opposed by Christman (7). In rusts without an aecium a very similar fusion occurs in the first uredinal generation (8) and in certain of the micro- and lepto-forms fusion occurs at the base of the telium (24). Moreover, the fusion is not necessarily between the basal cells which give rise directly to spores, as it may occur several cell generations earlier in the vegetative mycelium (24). Morphological evidence of the plasticity of the rusts is given by Dietel (9), who traces the derivation of reduced types from the corresponding heteroecious or autoecious forms. The teliosori of the reduced species occur in small or large groups like the *aecia* of the corresponding heteroecious type and bring about the same deformation of host tissues.

In an Endophyllum, Hoffmann (18) found an aecium of normal appearance producing aeciospores which germinated like teliospores by producing promycelia the sporidia of which lead directly to more aecia.

Recent work by Kunkel (21, 22, 23) and Dodge (12, 13) on the orange rusts of Rubus shows that side by side with the regular long-cycle rust (O, I, III) there occurs a short-cycle form in which the aeciospores germinate by forming promycelia, thus returning directly to the uninucleate condition and eliminating the telium. Moreover, according to Dodge (13, p. 494), there is some evidence of a "third or intermediate type which is maturing aecidia of two kinds," and which "may represent a strain of *Gymnoconia* which is particularly unstable and from which a short-cycled rust is now arising and which will have distinct morphological characters of its own."

These instances show the plasticity of the rusts in being able to shift the processes of fusion and reduction from one part of the life cycle to another, and the persistence of the processes themselves under varied conditions and alterations of the life cycle. Isolated instances occur where both fusion and reduction appear to be lost, as in the Endophyllum whose mycelium and spores are all uninucleate (26) and the uninucleate microform *Uromyces Rudbeckiae* (30), but these are exceptional.

Nor is such plasticity limited to rusts. In *Aspidium falcatum*, an apogamous fern (1), the sporophyte arises by a vegetative outgrowth from the prothallium without a fusion of nuclei. The "haploid sporophyte" so formed is normal in all morphological respects. In the sporangium 16 spore mother cells form, as in other ferns. These fuse by pairs to form 8, which then undergo reduction divisions, forming a maximum of 32 spores. Here the sexual process is shifted to the opposite end of the life cycle.

It would be interesting to know whether the aberrations in nuclear behavior observed in *Puccinia triticina* physiologic form 11 are to be regarded as an expression of the need of, and an ill-defined attempt at compensation for, the excised part of the life cycle. Also whether ultimately a revised nuclear history is to be expected, such as has been achieved in other rusts and by means of which *P. triticina* would be rendered independent of any aecial host.

Rusts represent the extreme of obligate parasitism. The success of the rust varies directly with the vigor of the host. The food of the fungus is obtained by means of haustoria which invade the living host cells. When the death of host tissues occurs in the infection the rust is weakened or even killed, for it can not subsist on decaying tissues. Susceptibility thus depends, for one thing, upon the ability of parasitized tissue to live and continue functioning more or less normally in company with the fungus.

Infections of *Puccinia triticina* physiologic form 11 on Little Club present an almost perfect picture of susceptibility, and this varies little with the age of the host. The fungus produces the minimum of disturbance in the host tissues. Even the first cell attacked survives, although on other hosts slightly less susceptible the first cell invariably succumbs. The walls of host tissues are not disintegrated, and the tissues are not plasmolyzed. The invaded tissues have somewhat heightened turgor, and their nuclei are slightly expanded. Under conditions favorable for the growth of the host, starch formation

proceeds at such a pace that the plastids have a normal or slightly greater than normal content of starch, in spite of the inroads of the fungus.

The presence of normal starch content in infections when host and rust are growing vigorously and apparently well adjusted to each other perhaps was to be expected. The presence of excess starch in infections on weakened leaves was a surprise. The leaves of this set received more sunshine, but this can hardly explain the difference, for uninfected tissues in 1924 and 1925 had similar starch content. (Table 2.) A possible explanation is that the fungus stimulates the photosynthetic activities of the host. Under good conditions, the excess starch is carried away. Under unfavorable conditions, some phase of the machinery of translocation breaks down and the starch accumulates. This excess starch apparently is utilized by the fungus; at least it is absent where the fungus is densest in old infections and is to be found then only in the marginal regions of the infection.

Rand's observations on pecan rosette (31, p. 25, 26) afford an interesting parallel in a widely different type of disease. Healthy leaves contained a moderate amount of starch at sundown and were starch-free the next morning. Mottled leaves showed "profound derangements * * * in starch assimilation and translocation." He says: "Mottled leaves of all sizes collected at night showed the green portions gorged with starch * * * swollen as if almost bursting with their accumulation of starch * * * Mottled leaves collected before sunrise the following morning appeared the same as those collected at night." It would be difficult to say what there is common to the two cases, save a possible breakdown in translocation.

SUMMARY

Puccinia triticina Erikss. is a heteroecious rust with uredinia and telia on wheat, and with aecia on exotic species of *Thalictrum*. In America the rust exists mainly if not exclusively as a continuous series of uredinial generations.

Puccinia triticina physiologic form 11 enters Little Club wheat freely. Forty hours after inoculation only 37 out of 204 fungi (about 18 per cent) still remained outside. Two appressoria at the same stoma may become united into one, and, when the two enter separately, the substomatal vesicles may fuse. About 4 per cent of the fungi studied showed this fusion.

In general, uredinial generations of rusts are made up of binucleate cells and are considered sporophytic. In *Puccinia triticina* physiologic form 11 the urediniospore has 2 nuclei, the appressorium usually 4, the substomatal vesicle commonly 8, the first hypha, after forming a haustorium, has 6 nuclei, one or two succeeding hyphae 4 or 5, and in all the later vegetative cells the number 3 predominates. Haustorium mother cells have 3 nuclei and haustoria have 1.

At the onset of reproduction the subepidermal mycelium concerned with spore production becomes binucleate. Spores, stalk cells, and the cells immediately below them regularly contain two nuclei.

Two cases of the fusion of hyphae were noted in the region where spore formation is imminent.

It is suggested that the irregularities in the nuclear history may have some connection with the prolonged growth of the rust in the absence of the aecial host.

Little Club wheat is fully susceptible to *Puccinia triticina* physiologic form 11. On Little Club wheat the fungus attains its maximum development, the haustoria expand fully, and spores are produced in abundance. The host tissues live, their cell walls (except in the guard cells at the stoma of entry) are not damaged, and the cell contents show a minimum of disturbance or impoverishment. The cells in the infection are more turgid, and their nuclei expand somewhat and move over to the haustoria. At the period of greatest activity of the fungus the plastids show little reduction in size. Under unfavorable conditions they may be temporarily gorged with starch. Not until extreme old age of the infections, do more than 1 or 2 per cent of the host cells die. In the more massive fungous growth on older host plants a few subepidermal mesophyll cells are crushed by the fungus. On the whole, the picture is one of excellent congeniality.

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EFFECT OF TEMPERATURE AND MOISTURE ON NEMATODE ROOT KNOT¹

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INTRODUCTION

Root knot, one of the most destructive of plant diseases, seems to be limited in its distribution largely by climatic conditions. Comparatively little work has been done on the fundamental problems related to the influence of temperature and moisture on the disease and its causal organism. This paper deals with experimental work on some of these problems; and the writer believes that some of the results of this work may be of practical economic importance to commercial greenhouse men and truck growers in the curtailment of losses due to root knot in celery, lettuce, and other low-temperature-loving plants.

THE DISEASE

Root knot attacks a wide range of host plants representing several hundred distinct species. These include practically all the common vegetables, many field crops, and even some fruit trees. The principal symptoms are enlargements or galls on the roots, varying in size from a pinhead in mild cases to a walnut where infestation is severe and complicated by the development of new generations of the parasite within the host near original points of infection. The causal organism is the nematode or eelworm *Heterodera radicicola* (Greef) Müller, a minute worm-shaped animal scarcely a millimeter in length at maturity. The galls formed as the result of stimulation of the host by the presence of the organism or some product excreted by it interfere greatly with the normal functioning of the roots; and the nematodes, which are ordinarily present in enormous numbers, feed entirely upon the host plant juices. These two effects together may seriously influence the metabolism of the host plant. In severe cases the plant is very much dwarfed or even entirely killed.

RELATION OF CLIMATE TO DISTRIBUTION OF ROOT KNOT

The nematodes are readily carried in soil clinging to bulbs or roots of plants, as well as within such plant tissues. They have been distributed widely throughout the country, north as well as south. Except in greenhouses, however, the disease is limited in its serious agricultural importance in the United States to the Southern and to some extent to the border States. It is at its worst in the extreme South. It is recognized as a factor in agriculture, however, in

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² The writer wishes to acknowledge his indebtedness to L. R. Jones and A. G. Johnson for their interest and suggestions throughout this work, and for the use of greenhouse and laboratory facilities of the department of plant pathology, University of Wisconsin.

Virginia, and reports are more and more frequent of its occurrence in Maryland, Delaware, and New Jersey. Southern Ohio, Indiana, and Illinois are finding it extensively in truck gardens and nurseries. Most of the Mississippi Valley States from Iowa northward do not have root knot to combat. Only occasionally is it to be found in small areas which are unusually favorable. In the northern tier of States in this section it is unknown outside the greenhouses. West of the Rocky Mountains, in Idaho, Oregon, and Washington, the disease is occasionally found.

The limiting factor on distribution seems therefore to be principally that of climate. Temperature may have its effect in two ways: (1) In its limitations on the actual life of the organism causing the disease, and (2) in its effect on the number of generations that may be produced even if the organism can remain alive throughout the year.

The earliest American literature on the subject recognized, in a general way, the limitations placed by severe winters on the distribution of root knot. Neal (6) stated that "the question of temperature is no doubt one of great importance in determining the boundaries of this disease, perhaps more so than food plants or soils." He stated that soil that is annually frozen from 6 to 10 inches is rendered practically free of the worms, except where they occur on perennial roots. Below 50° F. the worms were inactive, "paralyzed by cold." He cited the occurrence of the disease only in the South Atlantic and Gulf Coast States. Atkinson (1) referred to accounts of the disease in Germany, Java, Brazil, and in Scotland. He emphasized this latter occurrence as evidence against Neal's limitation of 50° F. winter temperature, and prophesied that if the pest once became established it would probably thrive as far north as the January isotherm of 35° F., which crosses Virginia and Tennessee. Marcinowski (5) included in the list of countries where the disease occurred (besides the central and southern European countries) Brazil, Chile, South Africa, Egypt, Madagascar, Sumatra, and Java, mostly countries with warm climates. Stone and Smith (7) considered freezing fatal to the root-knot nematode. Bessey (2), on the other hand, found root knot in northern Indiana, Michigan, and Nebraska, and considered that the nematode was able to maintain itself in regions where the winter's cold was very intense.

Aside from the question of freezing out in the winter, however, in the Southern States the long warm summers combined with favorable soil and moisture conditions bring about the development of several generations in a single growing season, thus greatly increasing the nematode population of the soil. Farther north, the shorter summer season shortens the period during which the nematodes can be active and therefore limits their increase.

Moisture has its effect principally in that it limits the growth of suitable host plants. Root knot occurs on many weeds in uncultivated lands in Georgia, Florida, and South Carolina, where there is continuous summer rainfall. In the Southwest, on the other hand, it is never found except in irrigated fields, the limiting factor being moisture and host availability, and not temperature.

Neal (6) stated that an excess of moisture was conducive to the disease, and that drainage was a remedy under such conditions. He also stated that moisture was essential to the vigorous growth

of the nematode, although it withstood an enormous amount of drying. Stone and Smith (7) agreed in general as to the need of moisture, and even said that nematodes do not seem to suffer from an excess of it, but stated that neither the nematodes nor their eggs can stand desiccation. In their experiments, soil in jars was allowed to dry out for a year, and at the end of that time the nematodes were not alive. Bessey (2), Frank (3, *pl. III*), and others conducted experiments which indicated that the larvae of the nematode were readily killed by drying. Excess of moisture, according to Bessey, does not seem to inhibit the organisms, unless the land is completely flooded and the organisms are loose in the soil and not protected by the roots of perennial plants. The flooding must be for at least 25 days to be efficacious in destroying the nematodes.

Considerable observational information is given in the papers cited, some of it more or less contradictory. Very little actual experimental work has been described, and practically no clear-cut conclusions reached. The subject of the influence of specific moisture conditions and temperatures on the development of the disease is left practically untouched. The present paper gives the results of definite experimental work which has cleared up conclusively some of the doubtful questions with regard to the relation of moisture and temperature to the development of root knot.

EFFECTS OF SOIL MOISTURE AND SOIL TEMPERATURE ON DEVELOPMENT OF ROOT KNOT

EXPERIMENTAL METHODS

Specific experiments for the purpose of determining the effects of soil moisture and soil temperature on the development of root knot were conducted at Madison, Wis., and Washington, D. C., in 1921, 1922, and 1923.

THE SOIL

Throughout the history of observations on root knot under field conditions, it has been known that the disease is usually at its worst in sandy soils. Consequently, sandy soil was determined upon at the outset for this experimental work. Usually virgin soil, composed largely of peat to which some manure was added or rich compost ordinarily used in the greenhouse, was made the basis, and to this about half the same volume of sand was added. This gave a rich sandy soil very favorable to the development of the root-knot nematode. Inoculation of the soil was accomplished by adding quantities of knotted roots of plants, found upon close examination to contain an abundance of eggs and larvae of *Heterodera radicumicola*. Figure 1 shows the lower part of a French cocklebur plant (*Urena lobata* L., a weed common in Florida, highly susceptible to root knot), which was used for a large number of the inoculations.

MOISTURE CONTENT

Standards for the determination and maintenance of soil moisture content were determined (1) by sampling in triplicate the thoroughly mixed soil and by oven-drying at 100° to 105° C., determining the dry weight, and (2) at the same time the percentage of moisture on the dry-weight basis necessary for complete saturation was determined, also in triplicate, and the average determined. The desired



FIG. 1.—Root of French cocklebur (*Urena lobata* L.), badly infested with root knot. Material such as this was used as source of inoculum for much of the soil-moisture and soil-temperature work. The larger discolored knots contain myriads of eggs and larvae of the root-knot nematode.

moisture in percentage of saturation moisture was then easily determined. For the early part of the soil-temperature work, a medium amount of moisture, usually 50 or 60 per cent, was maintained, this having been known from general observations to be favorable both to plant growth and development of the nematodes.

TEMPERATURE CONTROL

Soil-temperature control was accomplished by the use of the "Wisconsin soil-temperature tanks," a history of the development of which has recently been written by Jones (4). A new set of six tanks was built in the fall of 1921 for this purpose. The latest improvements were incorporated in them. During the course of the experiments, records were taken of the temperatures twice a day, morning and evening, and any irregularities were corrected at those times.

The readings were taken at a depth of about 5 inches in the water tanks. Variations of from 1 to $1\frac{1}{2}$ degrees were noted in different parts of a tank, which contributed, of course, to the inaccuracies of recorded temperatures. No device was used in any of these experiments to circulate the water in the tanks.

In general, temperatures within 1° C. in either direction were maintained regularly. Any great discrepancy was due to either the failure of the set of batteries which controlled the relays, thus allowing the temperatures to rise above the desired point, or to the burning out of a fuse controlling the heaters, which permitted the temperatures to fall. When anything of the kind happened, the correction was usually made within a few hours of the break, so that for practically the entire length of the experiments the temperatures averaged within the desired range for each tank.

DURATION OF EXPERIMENTS

Experiments were continued as a rule for a 30-day period. Under the most favorable conditions this permitted the development of only one entire life cycle, from egg or larva in the soil to infection and growth to the adult stage within the host plant. Consequently one condition was completely comparable with another in the matter of primary infections. No secondary infections, from the young of matured individuals developing under the most favorable conditions, entered into the results, except in those experiments in which the duration of exposure was definitely longer than 30 days.

MANNER OF RECORDING RESULTS

The results of a series of differences in one controlled environmental factor were to a certain extent evident in the tops of the plants. Figures 2 and 3 show these differences strikingly in the case of moisture control, the one in effect on the growth of the plants, and the other in relative growth under specific conditions as compared with the controls under the same conditions. Figure 4 shows the differences where the condition controlled is the temperature.

However, since considerable irregularities exist between different individuals under the same conditions, this is obviously not a sufficiently reliable basis for drawing conclusions on the effect of

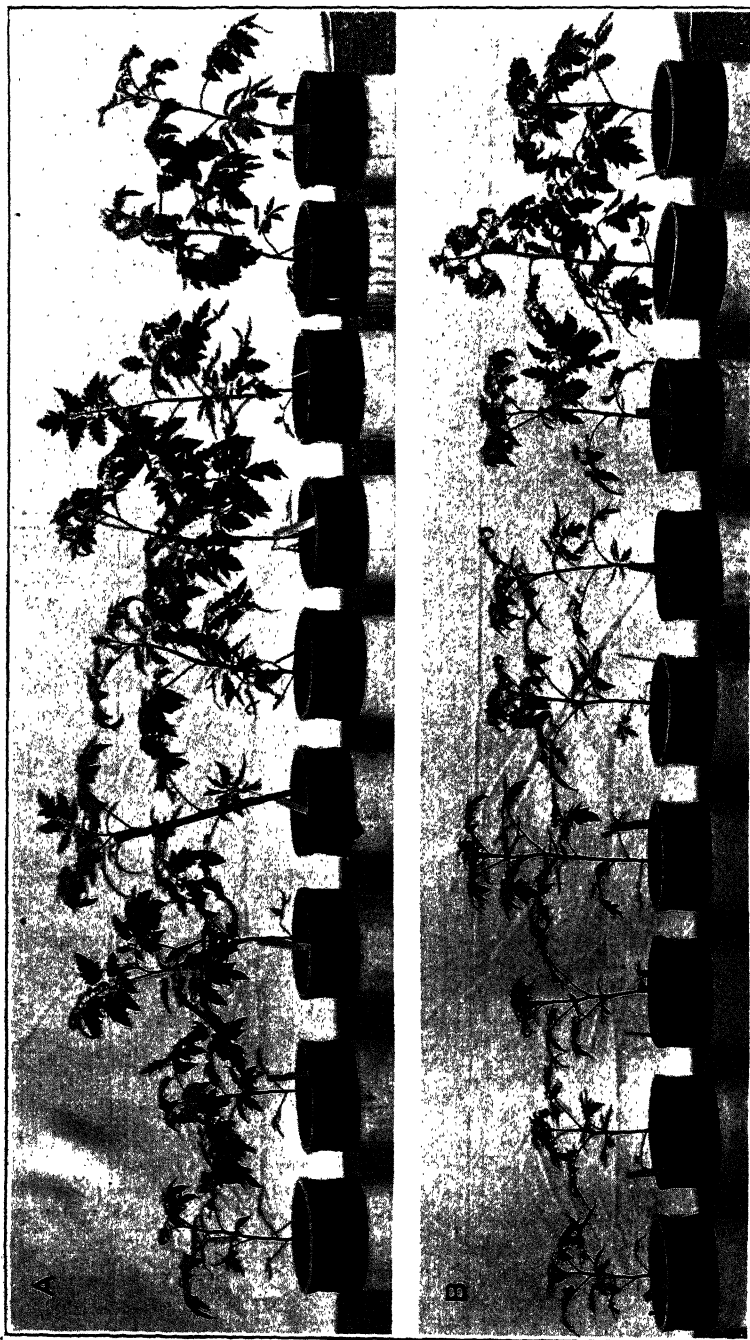


FIG. 2.—Tomato plants at the close of the controlled soil-moisture experiment 2
(For explanatory legend see page 229)

environmental factors on root knot. The most accurate way would be to determine the actual number of larvae that penetrated the roots under each condition. While this was perhaps possible, it was impracticable. The numbers under some conditions were so large and the difficulty of getting the organisms out too great to permit of it being done. It was decided, therefore, to make comparisons on the basis of number of galls per pot. Under some conditions infections were comparatively few, and individual galls could be counted very readily. This count was accomplished, as a rule, by placing the entire roots, which had been carefully removed from the soil at the close of an experiment and washed free from adhering dirt, into a large glass dish of water, over a black-surfaced table. Then with a pair of tweezers, observations could be made on each root separately, and counts made with an automatic counter. Under somewhat more severe conditions, galls were so numerous as to produce a beaded effect on many of the roots. Here again counts could be made very readily. Under still more severe conditions, however, galls coalesced, and individual counts could not readily be made. It would certainly not be legitimate to count a very small gall as the equal in value of a very large one, one possibly 20 times the size and containing many nematodes. It was arbitrarily decided in such cases to count each millimeter of the elongated swelling as one gall. As a rule, this appeared to give results roughly comparable with those of other conditions. In several cases even this means did not give results that reliably indicated the actual effect of root knot on the host plant. Some plants that were obviously severely injured gave a smaller total of knots than some that were less severely injured. In some cases, therefore, the results are given also in number of galls per unit of length of root. The total linear measurement of roots was taken, only the larger ones being measured. Very fine roots less than 2 centimeters in length were ignored. This method seemed to indicate accurately and reliably the relative injury done under the different conditions.

EFFECTS OF DIFFERENCES IN SOIL MOISTURE ON DEVELOPMENT OF ROOT KNOT

Aside from effects of flooding and of drying on the life of the root-knot nematode, only general observations have heretofore been made on the effect of degree of wetness of the soil on the development of root knot. These observations have indicated a relatively wide range within which the nematode is active in attacking the host plant. It was deemed desirable to make tests to determine definitely the range of activity of the organism in this respect.

EXPERIMENT 1

A preliminary experiment was conducted at Madison, Wis., in March, 1922, using tomatoes and soy beans. The usual sandy loam

EXPLANATORY LEGEND FOR FIGURE 2

A.—The control series, grown in sterile soil, moisture contents from left to right being 28 (approximate), 30, 40, 50, 60, 70, 80, 90, and 100 per cent of saturation. Very little growth occurred during the period of the experiment (one month) in the first two. Good vigorous growth occurred in the rest of the series, not much difference in growth being evident in the tops of the plants.

B.—The inoculation series arranged in the same order as the control series, and with exactly the same reduction. Note the greatly decreased vigor of the plants in the middle of the series.



FIG. 3.—Tomato plants grown for a period of two months under controlled soil-moisture conditions

A.—Forty per cent of the moisture-holding capacity, two inoculated and one control.

B.—Sixty per cent of moisture-holding capacity, showing even greater contrast between the inoculated plants and the control. The plants are somewhat larger at 60 than at 40 per cent.

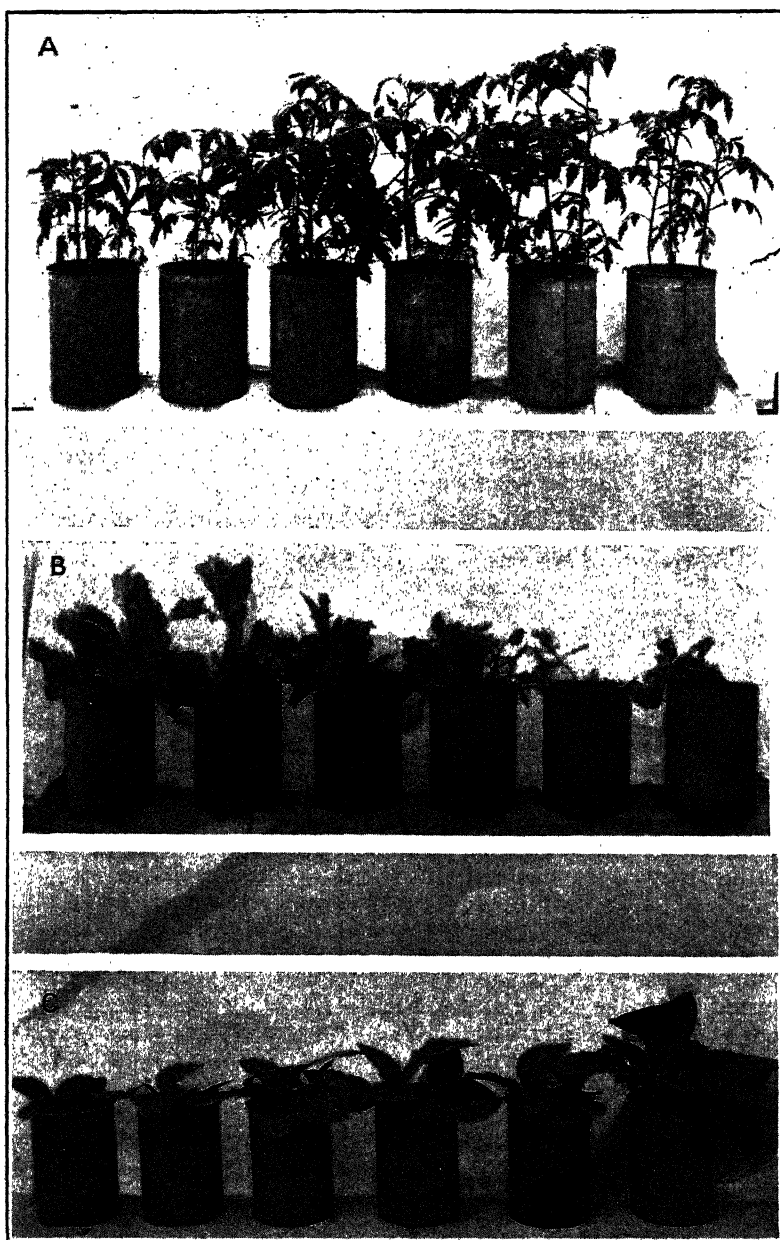


FIG. 4.—The effect of temperature on the growth of various plants

A.—Tomatoes grown in experiment 3, the temperatures, from right to left, being 15°, 20°, 25°, 30°, 35°, and 40° C.

B.—Lettuce from the same series, showing strikingly the reversal in effect of temperature from that shown in A. Soil fungi were partly responsible for this, however.

C.—Tobacco grown in a cool house, at soil temperatures of 10°, 13°, 16°, 19°, 22°, and 25° C, respectively.

(greenhouse compost plus sand) was used, lightly inoculated with nematode-infested dasheen material from Florida. The moistures maintained were 40, 50, 60, 70, and 80 per cent of the moisture-holding capacity of the soil. The lowest contained sufficient moisture to maintain plant growth to good advantage, and the highest was only slightly too wet. Water lost by transpiration and evaporation was replaced twice daily, morning and evening. One series was inoculated soil, and another series control.

The results were irregular and indicated the necessity of repeating the experiment under better conditions, particularly with a longer series of moistures, and with a larger number of inoculated plants under each condition. The results of experiment 1 are not recorded here.

EXPERIMENT 2

An experiment incorporating these desiderata was started at Washington, D. C., about November 1, 1922. Glazed crockery jars of about 2 gallons capacity were used. A sandy loam consisting of about two parts of compost and one part of sand was sterilized in the autoclave, then set out to aerate for several days in a large box. After a thorough mixing, samples were taken in triplicate for determining moisture content and moisture-holding capacity. Then nine jars were filled with even weights of soil, these to be the controls. Nematode material was added to the remainder of the soil, in the form of shredded and broken up badly diseased roots of French cocklebur (fig. 1) from Brooksville, Fla. The material contained an abundance of living nematodes, mostly in the larval stage. Twenty-seven jars were filled with this material to the same weights as the controls. This allowed for three inoculations and one control for each of the following percentages of moisture-holding capacity: 10, 20, 30, 40, 50, 60, 70, 80, and 90.

Vigorous young tomato plants were set out at once. They were permitted to stand under equal conditions for two days, while the moisture content was being determined. The content at time of planting was approximately 40 per cent of water-holding capacity. At the end of the two days the higher moistures were made up by adding the requisite amounts of water. It was 14 days, however, before sufficient moisture was lost from the lower pots to reach the 30 per cent mark, and the 20 and 10 per cent points were never attained. On the 15th day from the start, the 10 per cent jars were made over to 100 per cent, or complete saturation. The moisture content of the 20 per cent jars at this time was somewhat lower than that in the 30 per cent jars, and the 20 per cent jars were maintained throughout the experiment without the addition of any water.

Several extra jars were planted at the time the experiment was started and maintained at 40 and 60 per cent of saturation. These were utilized for the determination of accrued weights brought about by plant growth from week to week, these weights being added to the various jars to make up for the substance of the plants themselves. The accrued weights were estimated for pots other than those containing the 40 and 60 per cent moistures. The total accrued weight by the third week did not exceed 20 grams, so estimates were well within the allowable divergences. (The aim was

to keep weights within the range of 5 per cent below the percentages fixed upon. Thus, when the 60 per cent jars first lost enough moisture (76 grams) during the course of 24 hours to make it 55 per cent at its lowest point, water was added to bring it up to the desired weight twice daily thereafter; and similarly with the rest of the jars.) The jars were arranged on the greenhouse bench four deep from front to back, and were rotated in their relative positions with reference to light by shifting the front rows to the back and bringing the rest forward once each day. Thus all had approximately equal environmental conditions aside from moisture. Greenhouse temperatures ranging from 15° C. at night to 20° C. in the daytime were maintained.

On December 7, just 30 days after beginning it, the experiment was brought to a close. The effect of differences in moisture content was striking, especially in the control series. Very little growth occurred at 30 per cent of moisture capacity or below. From these points growth gradually increased with each rise, even up to 90 per cent, except for some variations due to individual differences at the intermediate points. Height is not a good criterion of "best growth," since at the highest moistures the growth is weak and succulent, and contains less ash than at the medium conditions. This fact is considered in drawing conclusions. Figure 2 shows the control series ranging from 25 or 26 per cent moisture to 100 per cent saturation. Differences between inoculated and controls at the same moisture content were also striking, especially in the middle of the series. This is seen by comparing Figure 2, A (the control series) with Figure 2, B (the inoculation series). Growth was very much reduced at from 50 to 80 per cent in the inoculated jars, while it was at its best in the controls. These differences are seen still more strikingly by comparing all three of the inoculated plants with the controls, as in Figure 3, A and B, showing 40 and 60 per cent jars, respectively.

As stated under "Methods," a comparison of the root systems entered into the ultimate results. Marked differences in type of root growth were noted. At the lower moistures roots were irregular, bent, gnarled, and in intimate contact with the soil particles, from which several washings would not free them. Root-hair development was prominent. At the higher moistures roots were smooth, straight, and clean, and root hairs were not evident (fig. 5, A). Differences in extent of root growth, as influenced by amount of soil moisture, were also prominent. In dry soil the roots extended to the bottom of the cans and became matted there, while in the moist soil they extended scarcely to the bottom. Fairly exact comparisons were made by measuring the total lengths of all the separate roots, except the very fine feeding thread roots and the short branches that were just developing. These measurements are recorded in Table 1. Typical roots from inoculated plants, as compared with controls, are shown in Figure 5, A, B, and C. Root-knot results are given in Table 1 in total number of galls per plant, and average number per plant for each condition. In addition, average numbers of galls per decimeter of length of root are tabulated. The graphs in Figure 6 bring out the same results more sharply. The table also shows the heights of plants in the entire series, in centimeters.

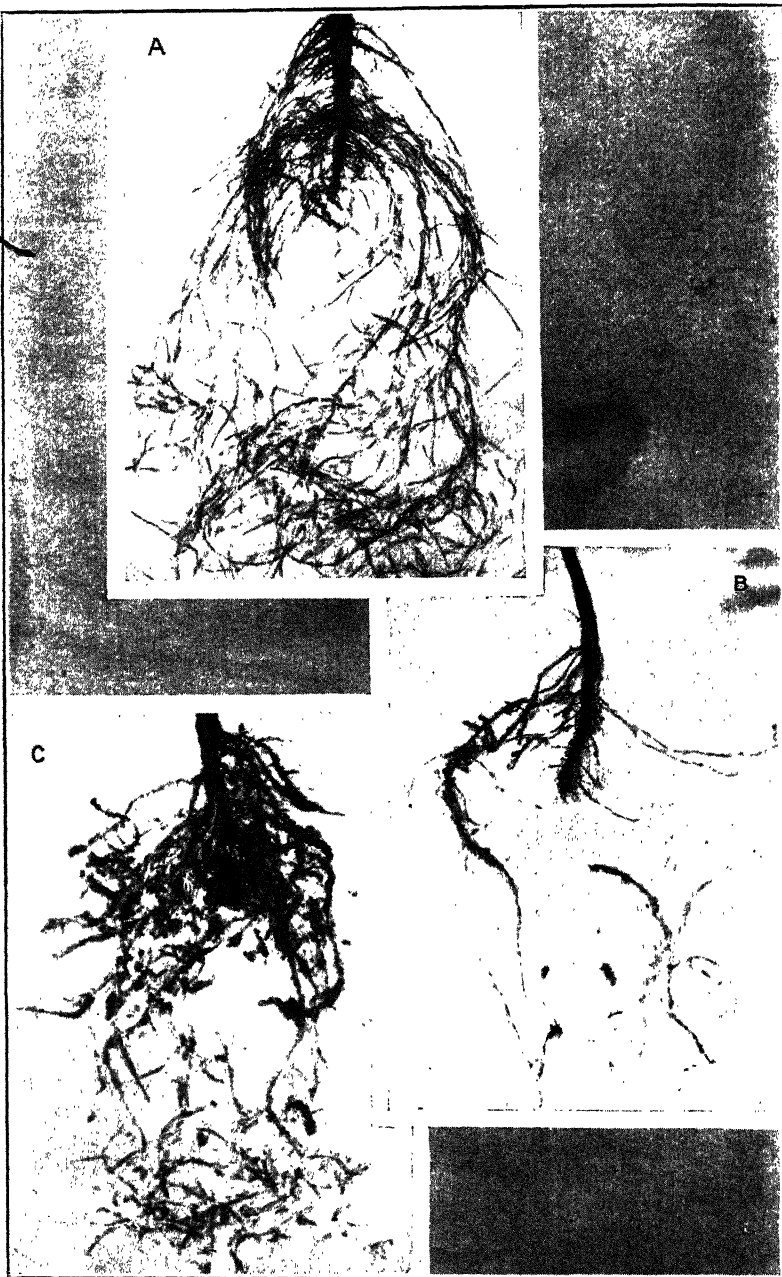


FIG. 5.—Tomato roots after two months in soil, at 60 per cent of the moisture-holding capacity
 A.—Root of control plant, clean and healthy.
 B.—Root of one of the inoculated plants, showing a large proportion of the root killed, owing to severity of root knot.
 C.—Root of the other inoculated plant, showing severe infestation, but the greater part is still alive. A comparison of B and C shows why a count of the number of knots on the roots is not an accurate criterion of the severity of infection.

TABLE 1.—Effect of soil moisture on root-knot development in tomatoes

Moisture (per cent of saturation)	Control		Series A				Series B			
	Height of plants	Length of roots	Height of plants	Length of roots	Number of knots	Number of knots per dm.	Height of plants	Length of roots	Number of knots	Number of knots per dm.
28 to 30 ¹	Cm. 27	Cm. 1,084	Cm. 22	Cm. 405	195	4.81	Cm. 30	Cm. 1,043	818	7.84
30 to 35 ²	30	1,098	20	309	787	25.46	30	565	418	7.36
40.....	41	32	706	1,257	17.80	27	520	959	18.44
50.....	50	805	28	343	705	20.55	35	840	1,463	17.48
60.....	51	35	889	1,786	20.08	35	1,040	1,859	17.82
70.....	45	809	32	598	1,864	31.17	32	478	823	17.22
80.....	37	628	36	781	2,136	27.34	33	227	152	6.70
90.....	37	492	45	1,046	1,366	13.05	44	666	1,181	17.73
100 ³	34	368	30	361	641	17.75	33	375	346	9.23

Moisture (per cent of saturation)	Series C				Averages of series A, B, and C			
	Height of plants	Length of roots	Number of knots	Number of knots per dm.	Height of plants	Length of roots	Number of knots	Average number of knots per dm.
28 to 30 ¹	Cm. 28	Cm. 611	1,088	17.48	Cm. 27	686±102	694±124	10.04±2.10
30 to 35 ²	31	543	643	11.84	27	472±45	615±51	14.89±3.00
40.....	33	750	1,617	21.55	31	659±39	1,278±28	19.27±.64
50.....	41	883	1,614	18.28	35	689±95	1,261±194	18.76±.51
60.....	39	663	1,681	25.05	36	864±60	1,769±37	21.00±1.28
70.....	37	410	593	14.46	34	495±30	1,093±186	20.95±2.55
80.....	35	551	1,124	20.40	35	520±88	1,137±273	18.15±3.34
90.....	50	868	1,394	16.06	46	860±60	1,314±31	15.61±.84
100 ³	40	398	243	6.11	34	378±6	410±57	11.03±1.92

¹ This did not get down to 30 until the experiment was half completed. No water was added during the experiment.

² Not down to 30 until experiment was half completed.

³ Changed from approximately 30 per cent half way through the experiment. No doubt much of the infection occurred before the change was made.

Table 1 shows, in the controls, a definite decrease in length of roots, as amount of moisture is increased. This regularity does not hold with the inoculated plants, where another factor, root knot, enters in. Figure 2, and the data for top growth in Table 1, show that saturation below 40 per cent and above 70 per cent are not favorable to the best growth of the plants. Within the range of best growth, and even above it up to 90 per cent, the root knot is at its worst. This is shown by both curves in Figure 6. At 70 and 80 per cent the average number of knots dropped off, but the number per unit of length of roots is highest of all, showing apparently greater

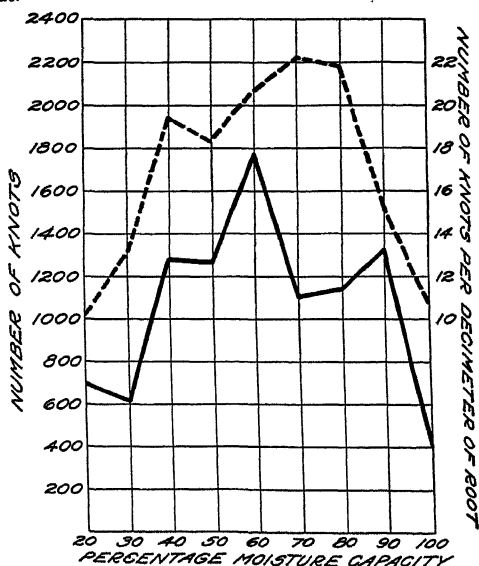


FIG. 6.—Effect of moisture on development of root knot in tomatoes. (The unbroken line represents number of knots; the dotted line, number of knots per decimeter of root.)

injury by the root knot there than at the other moistures. The number of individuals under each condition, in this experiment, was so small and the probable errors so large that not much significance can be attached to the differences shown between adjacent moisture contents. The general conclusion that within the range of best growth of the host plant the nematode is most active is obvious. This will probably hold true for most of the ordinary susceptible plants that are grown under root-knot conditions. The soil moisture, under growing conditions, fluctuates considerably, of course, but it is undoubtedly between 40 and 70 per cent of saturation the greater part of the time.

EFFECTS OF SOIL TEMPERATURE ON ROOT-KNOT DEVELOPMENT

Preliminary experiments on effects of soil temperature on root knot were conducted in the greenhouses of the department of plant pathology, University of Wisconsin, Madison, Wis., in the fall of 1921. These were followed by more conclusive experiments at Madison and also at Washington, D. C., during the early spring of 1922 and in the winter of 1922-23.

EXPERIMENT 3

The basis of the soil used in this experiment was greenhouse bench soil which had borne a crop of badly root-knotted cucumber during the summer. To this was added about half its volume of sand to improve its texture and make it more favorable for nematodes. Additional inoculum was added in the form of root-knot-infested cow-pea roots and dasheens from Florida. Control soil was made up from similar bench soil that had not borne root-knot affected crops. Moisture was maintained according to the usual procedure at 50 per cent of the moisture-holding capacity of the soil, which degree of moisture was chosen because of its obvious suitability for growth of the plants. The plants used were lettuce and tomatoes, three inoculation cans and one control of each per tank. Three healthy plants, from 2-inch pots, were set out in each can. Temperatures maintained were 15°, 20°, 25°, 30°, 35°, and 40° C. The daily records show no very great fluctuations from the desired temperatures during the period of the experiment, November 18 to December 18, 1922. On December 4 the tanks dropped from 2° to 5° (the higher temperatures dropping the most), and three days before the close of the experiment the highest tank went up 3°. Aside from these fluctuations, which lasted at most only four or five hours, variations from the desired temperatures were only 1½° or 2° and were corrected twice daily.

This experiment, with 5° intervals, was conducted in part for the purpose of determining cardinal temperatures for the growth of the plants. Results in vigor of growth in the case of the tomatoes are shown in Figure 4, A. Poorest growth resulted at the lower temperatures. Top growth increased with the higher temperatures, up to 35° C. At 40° the plant remained alive and even grew slightly, but very little root growth was made. In the matter of root growth, results were somewhat contrary to what might have been expected from the appearance of the tops. Roots were vigorous at the lowest

temperature (15°), occasional strands extending to the bottoms of the 9-inch-depth cans. At 20° root growth was somewhat more extensive, while at 25° and 30° it was very vigorous, with heavy roots matted in the bottoms of the cans. At 35°, however, root growth was much reduced, even though top growth was greatest at this temperature. At 40° there was practically no root growth outside the ball of the soil in which the plant was introduced into the cans.

Root growth in inoculation cans was approximately the same as in the controls.

Results in root-knot development were recorded in number of knots per can. The figures, including the averages, are given in Table 2. The average results are also shown in Figure 7.

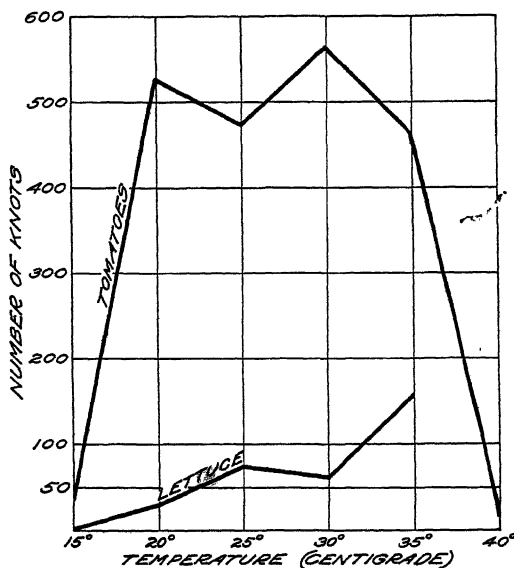


Fig. 7.—Effect of temperature on development of root knot in tomatoes and lettuce

TABLE 2.—Effect of soil temperature on development of root knot in tomatoes and lettuce

Tank No.	Temperature, degrees C.	Tomatoes			Lettuce		
		Number of knots			Number of knots		
		Plants in first can	Plants in second can	Average	Plants in first can	Plants in second can	Average
1.....	15	20	54	37 ± 8	0	4	2 ± .95
2.....	20	617	431	524 ± 44	25	33	29 ± 1.9
3.....	25	343	607	475 ± 63	105	41	73 ± 15.3
4.....	30	548	573	560 ± 3	-----	59	59
5.....	35	471	460	466 ± 3	152	-----	152
6.....	40	10	21	16 ± 2	-----	-----	-----

It is evident that at 15° C. the amount of root knot is negligible. It is very abundant at 20°, indicating a rather sharp point below which infection does not take place. From 20° up infection is abundant up to the upper limit of heat endurance by the host plant. The few knots that are recorded for 40° were near the surface, where the temperature was probably somewhat lower than in the interior of the cans.

In lettuce the effects of temperature on growth of the plants, as shown by the controls, was almost the reverse of what it was with tomatoes. By far the best growth resulted at the lowest temperature, and as temperatures increased growth and vigor of

the plants decreased. Another factor besides temperature entered into the case of lettuce. As already stated, the soil was not sterilized. As a result of the presence of the lettuce drop fungus, *Sclerotinia libertiana*, one of the root-knot-inoculated plants each at 30° and 35° C., and one at 30° in a control, were killed, and several others weakened. Figure 4, B, shows a series of lettuce plants in control cans, illustrating the striking decrease in vigor as the temperatures increase. The vigor of the roots correspond with that of the tops. At 15° C. roots were long and vigorous and became matted in the bottoms of the cans. At 20° they were only slightly less, but at 25° they were much reduced in length and fewer in number. At 30° they reached only about halfway to the bottom, and at 35° were only a few inches in total length. At 40° the plants did not survive.

Results in amount of root knot were possibly vitiated to a certain extent by the presence of the other organisms in the soil. They showed a striking increase with the temperature, however. Table 2 and the lettuce curve in Figure 7 show the marked effect of temperature on root-knot development with this crop. The same reservations on significance of results apply here as in experiment 2, because of the small number of individuals.

These results indicate that temperature is a limiting factor in root-knot development. They also indicate that in spite of the great differences in reaction of the hosts (tomato and lettuce) to temperature, the former being high-temperature-loving and the latter low, the results in root-knot infection were about the same. The need was evident for further experiments with a closer series of temperatures, and several different hosts.

EXPERIMENTS 4 AND 5

Using the same soil as before, increased in quantity by the addition of further compost and sand in the same proportions, and reinoculated with nematode material from Florida, a new series was started, as follows: Experiment 4 was conducted in a cool house, with six tanks, in which temperatures of 10°, 13°, 16°, 19°, 22°, and 25° C. were maintained. At the same time experiment 5 was started, in a warmer house, in seven tanks running 22°, 25°, 28°, 31°, 34°, 36°, and 38°. The plants used in the entire series were cucumbers and tobacco. Two cucumber plants were put into each of two cans in each tank, and two plants of White Burley tobacco into each of two more cans. The same series of temperatures was used for stem-nematode studies, so only the four cans were used for the root-knot organism. Since temperature and light conditions were markedly different in the two houses, the two experiments are considered as separate and distinct. No great fluctuations from the desired temperature or moisture occurred. The cucumbers did not grow well, possibly because of the season and shortage of light. In tank No. 1, at 10°, the first planting, and later a second one, succumbed completely to the unfavorable environment. Growth was increasingly better from 13° up to 25°. Root growth was fair, about 3 inches in depth, at 13°, and root knot was entirely absent. At 16° roots were somewhat better, and a small amount of root knot developed. At 19° root knot was abundant (figs. 8 and 9) on roots that extended in a few strands to the bottoms of the cans. At 22° roots and knots both were somewhat

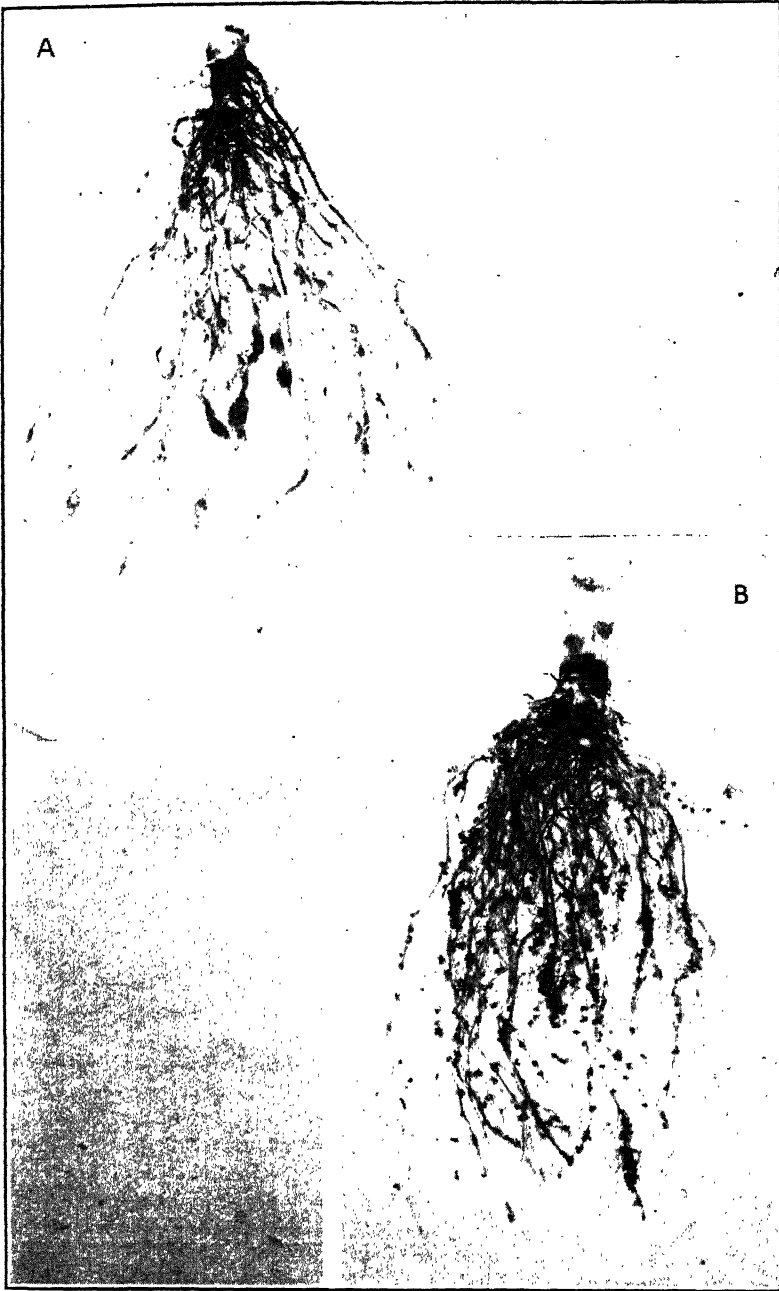


Fig. 8.—Roots of tobacco plants grown in root-knot-inoculated soil under controlled soil temperature conditions

A.—Root taken from a can held at 19° C., showing large galls.

B.—Root from the 25° C. tank, showing much more numerous, but smaller, galls, apparently burst out at the sides.

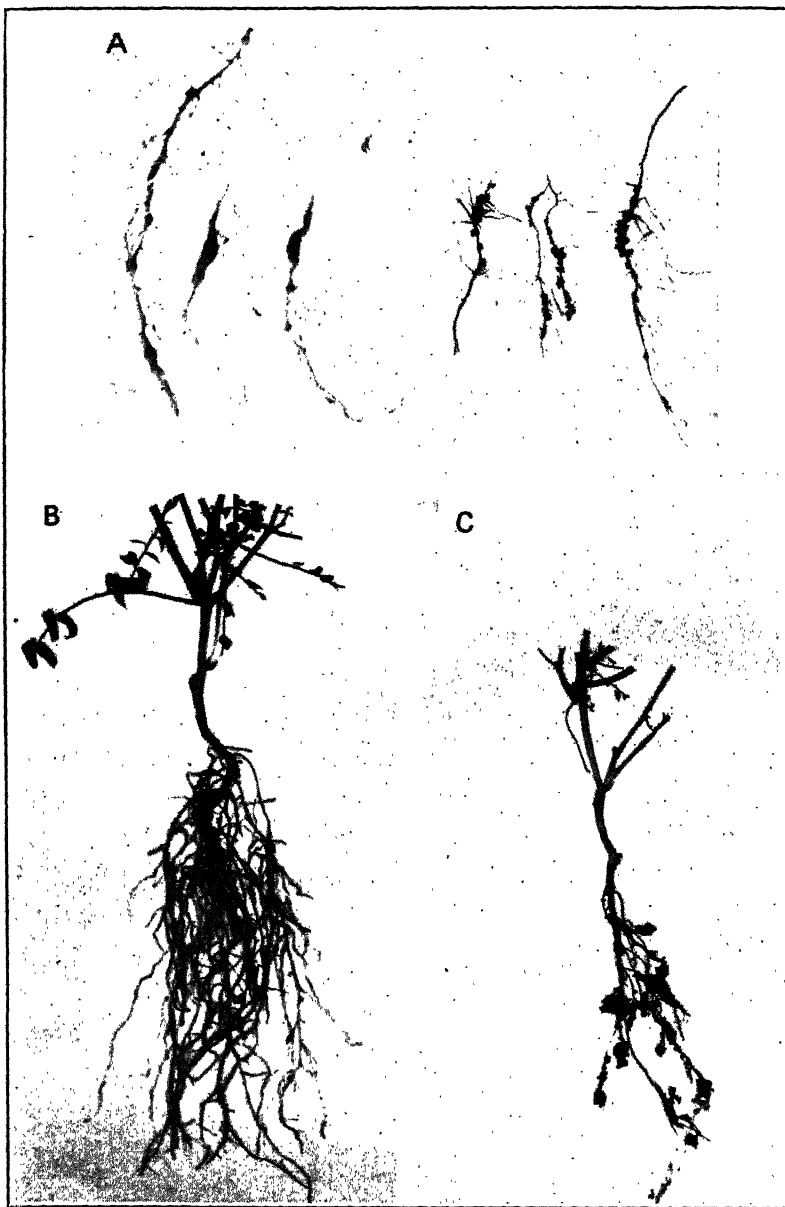


FIG. 9.—A.—Effect of temperature on type of root knots on tobacco: Left, Grown at 19° C.; Right, Grown at 25° C. B. and C.—Effect of temperature on development of root knot in vetch (*Vicia monantha*): B.—Plants grown at 12° C. Note the good nodule development, as well as the freedom from root knot. C.—Plants grown at 27° C. Note the poor root growth, abundant root knot, and total absence of beneficial nodules

reduced, but the amount of infection would appear to be an individual difference rather than a temperature reaction, for at the next higher temperature, 25°, the number of knots was double that of any lower temperature, and the roots were much reduced, due largely to the root knot. Table 3 shows the figures for amount of root knot, and Figure 10 shows the same results graphically.

The tobacco made much better growth than the cucumbers, and its reactions were such as to justify attributing them directly to temperatures. Figure 4, C, shows tobacco plants grown in tanks 1 to 6, and depicts clearly the relative growth at the different temperatures, there being a uniform increase from the low to high. Root-knot results were very striking, and, as shown in Table 3, bring out more sharply than does experiment 3 the critical temperature for development of the knots.

These experiments show that there is a critical temperature below which root knot does not develop. The differences in the tobacco plants between the averages at 16° and 19° C. are striking, and indicate that 16° or a trifle below that temperature is the dividing line between negligible

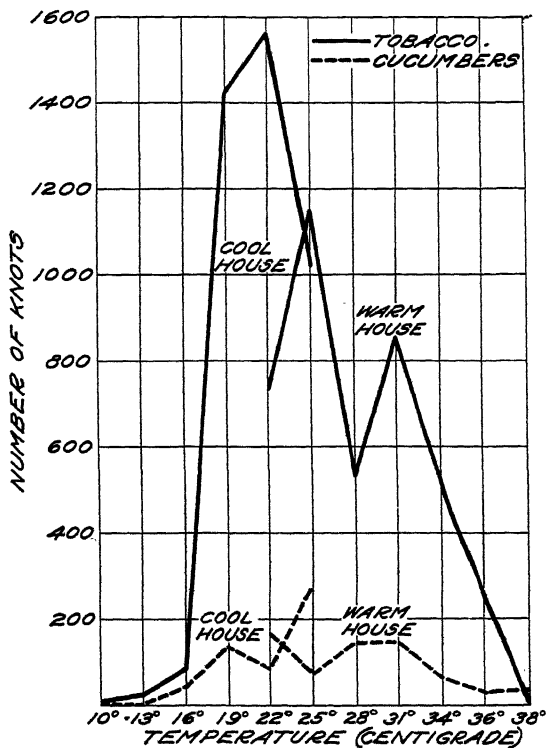


FIG. 10.—Effect of temperature on root-knot development in cucumber and tobacco

infection and abundant and injurious infection. The infections at 10° and 13° may have taken place in the three-day interval before the cans were placed in the tanks, when they were all standing under equal conditions to get an even start; or, again, they may have taken place in the surface region of the soil; for, as has been said, in warm weather the surface layer sometimes warmed up a trifle in the lower tanks. It would appear to be justifiable to conclude, however, that the small amount of root-knot development at the lower temperatures was due to either the inactivity of the nematode at these temperatures, or to a host reaction that was common to both high and low temperature loving plants. Of the two explanations, the former seems the more plausible. At the other extreme, severity of root-knot infestation appears to be limited only by the

limited plant growth. There was a distinct dropping off in infection, however, in both cucumber and tobacco between 31° and 34° C. Above about 36° very little infection took place in the tobacco, though it was still abundant in cucumbers.

TABLE 3.—Effects of soil temperature on root knot in cucumber and tobacco

COOL HOUSE					
Tank No.	Temperatures, degrees C.	Cucumber (number of knots)	Tobacco (number of knots)		
			Plants in first series	Plants in second series	Average
1.....	10	0	0	8	4± 1.9
2.....	13	0	7	23	15± 3.8
3.....	16	39	60	98	79± 9.0
4.....	19	134	1, 182	1, 634	1, 423±124. 5
5.....	22	83	1, 435	1, 683	1, 559± 59. 1
6.....	25	270	628	1, 423	1, 026±189. 3

WARM HOUSE					
1.....	22	165	339	1, 125	732±187. 4
2.....	25	71	1, 056	1, 266	1, 161± 50. 1
3.....	28	139	483	581	532± 23. 4
4.....	31	142	1, 138	563	861±137. 4
5.....	34	61	391	651	521± 62
6.....	36	26	247	303	275± 13. 4
7.....	38	31	-----	4	4

EXPERIMENT 6

In the latter part of February, 1922, an experiment was started with Early Buff soy beans. The cans were prepared as usual, and the

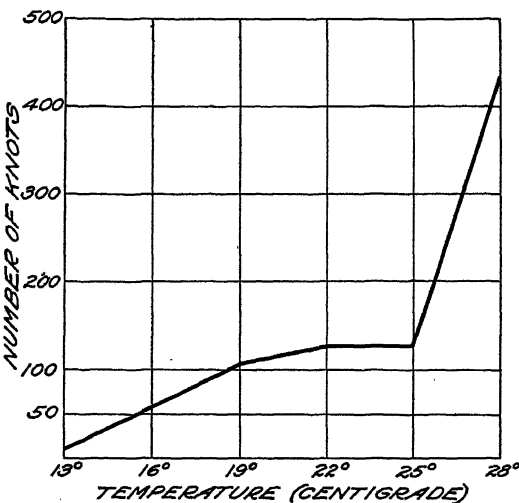


FIG. 11.—Effect of temperature on development of root knot in soy beans

bean seed planted, all the cans being held under the same conditions until they were well started. Approximately two weeks were required for this. On March 15 four inoculation and two control cans, each containing two soy-bean plants with the cotyledons fully developed into primary leaves, and secondary leaves just appearing, were placed into the tanks in a house averaging about 19° C. Soil moisture was maintained at 60 per cent of capacity. The temperatures of the tanks were started at 13°, 16°, 19°, 22°, 25°, and 28°, and held within a

degree or two of these readings throughout the experiment, which closed April 6.

The plants showed gradually increased vigor from the lowest to the highest temperatures. There was a distinct difference between the controls and the inoculated at the upper three temperatures, a slight difference at 19°, and none at all at the lower two. Root-knot development showed a distinct relation to temperature, as shown by Table 4 and Figure 11. Differences between adjacent temperature steps were sometimes not significant, but wider comparisons were usually distinctly so.

TABLE 4.—Effect of soil temperature on root-knot development in soy beans*

Tank No.	Temperature, degrees C.	Control			Inoculated									
		Plants in first can	Plants in second can	Average height	Plants in first can		Plants in second can		Plants in third can		Plants in fourth can		Average	
		Height	Height		Height	Number of knots	Height	Number of knots	Height	Number of knots	Height	Number of knots	Height	Number of knots
1	13	<i>Cm.</i> 12	<i>Cm.</i> 12	<i>Cm.</i> 12	<i>Cm.</i> 9	4	<i>Cm.</i> 10	10	<i>Cm.</i> 10	6	<i>Cm.</i> 10	3	<i>Cm.</i> 10	6 ± 1
2	16	11	12	12	10	45	12	75	13	48	12	55	12	56 ± 4
3	19	14	13	13	12	56	13	153	11	76	12	141	12	106 ± 14
4	22	16	14	15	13	225	13	31	13	128	14	117	13	125 ± 23
5	25	18	18	18	16	133	15	86	15	226	18	55	16	125 ± 22
6	28	22	23	22	19	186	18	669	21	502	19	321	19	420 ± 61

EXPERIMENT 7

Early in the temperature studies it seemed desirable to conduct an experiment in which a controlled daily rise and fall of temperature could be compared with certain constant temperatures. Preliminary work at Madison, Wis., with a fluctuating-temperature tank made it evident that the exact range of temperatures desired could be obtained very closely. Consequently, at Washington, in the fall of 1922, the control apparatus was improved through one or two stages until the apparatus represented by the drawing in Figure 12 was made. *A* is the spring from an ordinary 5-cent mouse trap. The different parts are secured in place on a slab of transite by means of strong copper wire at *a*, *b*, *c*, and *d*. Points *a* and *b* are connected, by means of this same wire, with the binding posts *b* and *c*, respectively. Since the spring extension *e* must conduct an electric current, it is wound with copper wire of about 24 gauge, thus connecting posts *B* and *C* when the trap is set. The trip *f* is connected by means of a very fine wire with the clapper of an alarm clock.

A simple battery switch *FGH* is located near by. The wires from the heater are

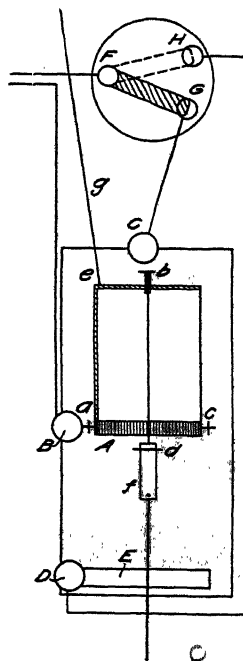


FIG. 12.—Diagram of the apparatus designed to control the temperature in a fluctuating-temperature tank

connected at point *F* on the switch, and at post *B* on the trap. *G* on the switch is connected by heavy current wire with post *C*, and *H* with post *D* on the trap. In the evening the switch connection *FH* is made, and the trap is set. The release of the alarm at the desired hour in the morning springs the trap. This makes the necessary contact on the copper plate (*E*) to close the circuit through the heater immersed in the tank. At the same time the pull of the spring is utilized to jerk out of its position by means of a string the outlet of the rubber hose carrying a stream of cold water, turning the water from the fluctuating tank to another outlet. The temperature of the tank immediately begins a gradual rise, therefore, until the desired upper limit is reached, when it is cut off by means of the usual thermostatic control.

Meanwhile, sometime during the day the trap and alarm are reset and the current switched from *FH* to *FG*. Now, when the trap is set the circuit is closed, instead of open as with the night setting; and when the trap is sprung at the desired hour in the afternoon, the contact made by the trap spring and tongue is broken, and the circuit is opened, thus immediately shutting off the heat in the tank. Again, the pull of the spring releases the small hose carrying the stream of water from its position during the day (where it has been held by a small plug in the pulley), so that it springs back and carries the stream into the fluctuating tank again. Thus the temperature immediately begins to fall, and continues to drop until the equilibrium of the city water temperature is reached. By running the stream of water fast or slow, as the need may be, a variation of two or three degrees in low temperature can be accomplished.

Experiment 7 was started on January 16, when three inoculation and one control cans each of lettuce and radish were planted. Three days later the cans were set into the tanks running temperatures as follows: No. 1, fluctuating, 10° to 19° C.; No. 2, 10°; No. 3, 15°; No. 4, 17°; No. 5, 19°; No. 6, 23°. Thermographic records were kept of water temperatures in the fluctuating, low, and medium temperatures by means of soil thermographs. Figure 13 shows specimen records for one week. Frequent tests showed that the soil temperature in the center of the cans followed very closely after the temperature of the water as indicated by the thermographs. The immersed bulb of the thermograph lagged somewhat behind the water itself, as did also the soil temperatures in the cans, so the record gives a fairly accurate representation of the changes that actually took place in the cans of soil. No great variations from the desired temperatures occurred in any of the other tanks. Moistures were kept up within five points of 60 per cent of the moisture-holding capacity of the soil.

It was not deemed profitable to make counts with the radishes, because of the unsuitability of the material for making this sort of record. The results from casual examination of the roots at the time they were removed showed that they followed very closely the results on the lettuce. No infection was evident at 10° C., a medium amount at 15° and the fluctuating temperatures, then an apparently increasing abundance at 17°, 20°, and 23°. The growth of the radishes was irregular, but apparently best at the medium and fluctuating temperatures, the roots being much better here than at any of the other temperatures.

The lettuce proved to be much the more desirable plant of the two for obtaining results on root knot. The response of the plants to the temperatures was somewhat as it was in experiment 3, though the higher temperatures were not nearly so hard on the plants, due to the fact that no injurious fungi were present in the soil. (Sterilized soil was used.) The plants grown at 15° C. and at the fluctuat-

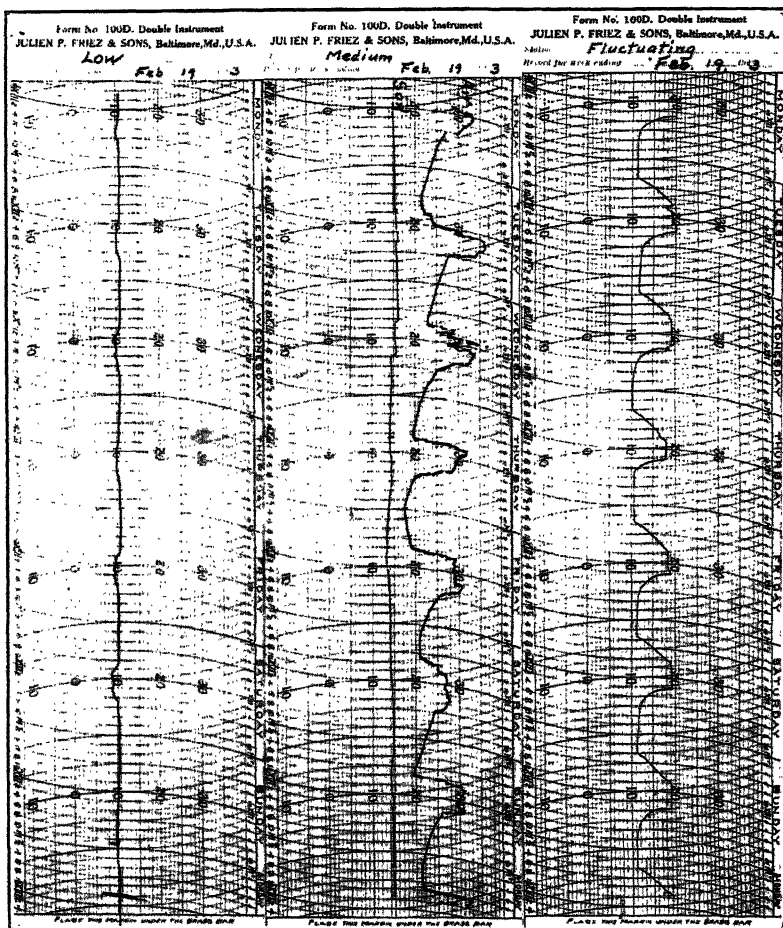


FIG. 13.—Record of temperatures in three of the tanks during one week of experiment 7, in the greenhouse at Washington, D. C. Note the fairly uniform daily range of temperature obtained in the fluctuating tank. The periodical drop indicated on the sheet marked "Low" was caused by the inflow of water from the fluctuating tank, when its temperature was automatically started on its rise. The middle sheet shows the air temperature, as well as the "Medium" soil temperature, for the week ending February 19.

ing temperatures presented much the best appearance at the close of the experiment. At 10° good heads were produced, but they were not as large as those in the 15° tank. It is probable that the optimum for growth is between 10° and 15°. At the higher temperatures up to 20° good leaf growth was obtained, but the plants had long internodes and were not of good commercial type. At 23° the plants were smaller, showing that that was above the range of good growth.

Unfortunately, the soil used in this experiment had lost a great proportion of its nematodes when the last series (from experiment 2) were removed. Results therefore lack the striking contrasts that are usually present when the parasites are present in abundance. Table 5 indicates the effect of the temperatures on the lettuce root-knot infection. Figure 14 shows the same results.

TABLE 5.—Effect of soil temperature on development of root knot in lettuce in three inoculation cans

Tank No.	Temperature, degrees C.	Number of knots on—			
		Plants in first can	Plants in second can	Plants in third can	Average
1.....	10-19	55	145	45	82±17.5
2.....	10	0	6	0	2
3.....	15	150	72	94	105±12.8
4.....	17	450	167	220	279±47.8
5.....	20	610	380	367	452±43.4
6.....	23	218	245	231	231± 4.3

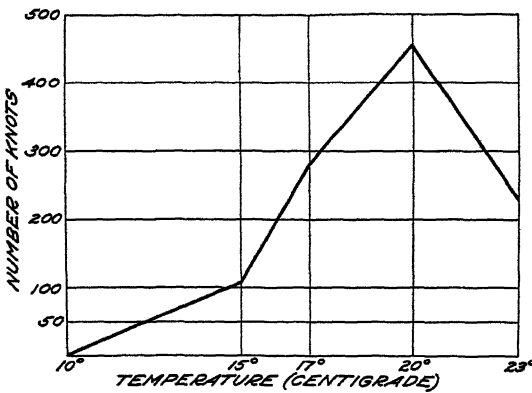


FIG. 14.—Effect of soil temperature on development of root knot in lettuce. The drop at 23° was due to reduced growth of the plant rather than to less favorable temperature

Table 5 and Figure 14 show practically no root knot at 10° C. and a greater amount with each rise in temperature up to 20°, as is to be expected from the results of previous experiments. The number of knots drops off at 23°, contrary to results with tobacco and tomato. This is to be expected, since it is in accord with the reduced growth of the host at that temperature. Root growth was much reduced, and

number of knots per unit length was undoubtedly greater here than at any other temperature. These data, however, are entirely misleading if total number of knots alone is considered as the criterion of severity of root knot.

In a comparison of the root knot in the fluctuating tank with that at the average or 15° tank, the record of temperatures should be considered first of all. While the maximum reached was quite consistently 20° C., and the minimum 10°, making an average of 15°, certain irregularities resulted from differences in the rising and falling curves of temperature. These differences are plainly evident in Figure 9. Although the hours of beginning of the rise and fall were just 12 hours apart, careful computation showed that during the course of the experiment the temperature was above the average for 295 hours out of the total period of 938 hours, or only about 31.5 per cent of the time. In accordance with what might be expected from this, the number of knots at the fluctuating temperature is

somewhat less than the number at the average, the difference being 23. The probable error of the difference is 21.5. The chances are about even, then, that the difference is due to chance rather than temperature. The difference is therefore not significant.

Further study of the record shows that the temperature was above 13° C., the approximate critical temperature for noteworthy infection, for 411 hours, and below it for 527 hours. The period favorable for infection was undoubtedly long enough to overcome the quiescence due to the cooler temperatures, and to permit development of definite symptoms of the disease.

EXPERIMENT 8

In order to test this point still further, a final experiment with the same apparatus was started March 5. The soil was thoroughly mixed and reinoculated. Roots of French cocklebur containing an abundance of live nematodes were used as source of inoculum. Vigorous young celery plants that had been held in 2-inch pots until roots were abundant and tops were about 4 inches high were used. Uniform plants were set out, one to a can, six in inoculated and two in sterile control soil for each of the six tanks. Moisture was maintained at approximately 60 per cent of moisture-holding capacity of the soil. Plants were inserted into the tanks after three days, to become established under like conditions. Tank temperatures were adjusted to 11°, 14°, 17°, 20°, 23° C., and fluctuating from 11° to 18°. The automatic control for the latter tank was set to start the rise in temperature about 2 o'clock in the morning and the fall at 4 o'clock in the afternoon. This was designed to compensate for the difference in shape of the rising and falling curves, and thus to make the periods more nearly even above and below the critical temperature for infection.

The experiment was brought to a close April 4, just 30 days after it was started. Fairly uniform curves were obtained in the fluctuating-temperature tank. Occasional irregularities occurred. During the experiment the temperature was above 13° C. (the critical temperature for infection) for 361 hours, and below it for 359 hours. It was above that of the "mean" tank (14°) for 283 hours, and below for 437 hours. The temperatures in the constant-temperature tanks did not vary greatly from the desired points. The results are shown in Table 6, in total number of knots, and in number of knots per decimeter of root length. In order to arrive at an absolute basis of comparison, these results were studied statistically, the deviations of the different plants of one lot from the mean were recorded, and the standard deviation and the probable error of the mean were calculated.

With these figures at hand, the actual significance of any specific differences can readily be seen. Comparing first the constant temperatures, Figure 15 shows to best advantage the uniform rise in amount of infection from 0 in the 11° tank, to nearly 11 knots per decimeter of root length in the 23° tank. Between the 11° tank and the 14° tank significance is self-evident, and verifies earlier results as to the critical temperature. Between the 14° tank and the 17° tank the difference, 2.28 knots, is sufficiently greater than the probable error of the difference, 0.33, to be significant, consequently it is justifiable to conclude that even in this short range

the stimulation of increased temperature has made itself felt in increased amount of infection. This stimulation appears to be continuous up to the highest temperature tested, 23° C. Between the 20° and 23° tanks the difference is 4.83 knots per decimeter. The probable error of the difference is 0.93, or less than one-fifth the difference between the two, consequently the uniformity of the curve of infection may be judged to be significant.

TABLE 6.—*Effect of temperature on root knot in celery*

Tank and can number	Temperature	Length of root	Number of knots	Knots per dm.
	° C.	Cm.		
Tank 1:				
Can 1	11	1,727	0	0
Can 2	11	2,345	0	0
Can 3	11	2,196	0	0
Can 4	11	1,022	0	0
Can 5	11	1,988	0	0
Can 6	11	1,648	0	0
Can 1 (control)	11	1,816	0	0
Can 2 (control)	11	3,586	0	0
Tank 2:				
Can 1	14	1,401	312	2.227
Can 2	14	2,371	377	1.590
Can 3	14	1,889	411	2.175
Can 4	14	1,364	429	3.145
Can 5	14	1,898	560	2.950
Can 6	14	1,881	505	2.684
Can 1 (control)	14	1,823	-----	-----
Can 2 (control)	14	3,228	-----	-----
Mean	-----	-----	-----	2.462±0.145
Tank 3:				
Can 1	17	874	379	4.336
Can 2	17	908	281	3.094
Can 3	17	1,208	503	4.181
Can 4	17	1,019	597	5.858
Can 5	17	1,785	842	4.717
Can 6	17	1,878	1,185	6.309
Can 1 (control)	17	1,270	-----	-----
Can 2 (control)	17	4,992	-----	-----
Mean	-----	-----	-----	4.749±0.295
Tank 4:				
Can 1	20	1,021	619	6.062
Can 2	20	1,195	627	5.247
Can 3	20	2,197	1,804	8.211
Can 4	20	759	466	6.139
Can 5	20	895	502	5.608
Can 6	20	1,376	787	5.719
Can 1 (control)	20	443	-----	-----
Can 2 (control)	20	6,431	-----	-----
Mean	-----	-----	-----	6.164±0.265
Tank 5:				
Can 1	23	1,024	1,270	12.402
Can 2	23	891	1,214	13.625
Can 3	23	665	927	13.939
Can 4	23	912	1,090	11.951
Can 5	23	488	219	4.487
Can 6	23	653	623	9.540
Can 1 (control)	23	1,126	-----	-----
Can 2 (control)	23	1,309	-----	-----
Mean	-----	-----	-----	10.991±0.891
Tank 6:				
Can 1	¹ 11-23	4,823	157	.325
Can 2	¹ 11-23	2,242	314	1.400
Can 3	¹ 11-23	1,067	144	1.349
Can 4	¹ 11-23	3,364	85	.252
Can 5	¹ 11-23	1,029	150	1.457
Can 6	¹ 11-23	2,242	218	.972
Can 1 (control)	¹ 11-23	1,261	-----	-----
Can 2 (control)	¹ 11-23	1,820	-----	-----
Mean	-----	-----	-----	.959±0.137

In comparing the plants grown in the fluctuating tank (12° to 18° C.) with those at 14° (just above the critical temperature), a significant difference is also observed, the difference of 1.49 knots being comparable with 0.20, a ratio of more than 7 to 1, which is well above the limits of what might be expected from random sampling. This is just as might be expected from the fact that the temperature was below 14° about 1.54 times as long as it was above, which shows again the rather delicate irritability of the nematodes to temperature as a stimulating agent. It shows that even several hours a day below the temperature at which they are capable of infesting a plant, does not render them incapable of infestation during the comparatively short period when temperature conditions are favorable.

EXPERIMENT 9

As a final experiment in the soil-temperature control series, tests were made with vetch, which is becoming more and more prominent as a winter green-manure crop in the South, and with potatoes. This series was started February 17, 1924, with rich sandy soil, steam-sterilized at first, then very heavily inoculated with fresh root-knot material of French cocklebur, *Urena lobata*, from Brooksville, Fla. In order to be certain of sufficient legume bacteria, a wet inoculation with a fresh culture of the vetch organism was made. The temperatures used were 12° , 15° , 18° , 21° , 24° , and 27° C.

The series was closed April 2. The temperature records, which were maintained throughout by means of soil thermographs, whose bulbs were immersed in the water at the average depth of the soil cans, showed in general very satisfactory control, except in the case of the low tank (12° C.), which occasionally ran too high. During the first week this tank was above 14° , the critical temperature for infection, for 10 hours. The third week it averaged more nearly 13° than 12° , and was above 14° for a total of 34 hours. In the fifth week it was up again on two occasions, for a total of 18 hours above 14° . The higher temperature was unavoidable; it was due to gradual rising of the temperature of the cold-water supply.

These 62 hours above the critical temperature for infection undoubtedly account for the infestation that took place in the potato roots in this tank. While some of the other tanks, notably those at 18° and 21° , showed an occasional wider fluctuation than was desirable, the averages were approximately as anticipated.

The results of this experiment are given in Tables 7 and 8, and are shown in Figures 16 and 17. (See also fig. 9, B, C.) Here again statistical methods are followed in order to obtain a reliable basis for comparisons.

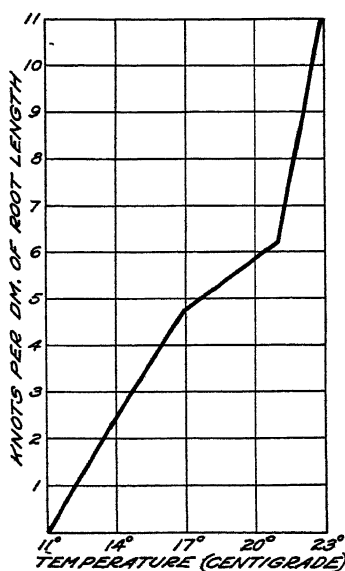


FIG. 15.—Effect of temperature on development of root knot in celery

The results with the vetch are particularly noteworthy, and show clearly the effect of higher temperature in increasing the amount of root knot within a given period. Figure 16 shows the gradual and almost uniform rise from none at all at 12° C. to about 20 per decimeter of root length at 27°. The effect is almost the reverse for infection of the roots by the nodule bacteria. At the lower temperatures (12°, 15°, and 18°) nodule development was abundant. At 12° it was much less and at 24° and 27° it was practically negligible.

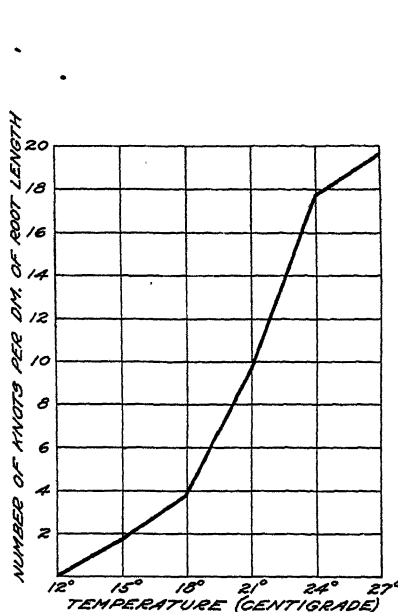


FIG. 16.—Effect of temperature on development of root knot in the vetch *Vicia monanthes*

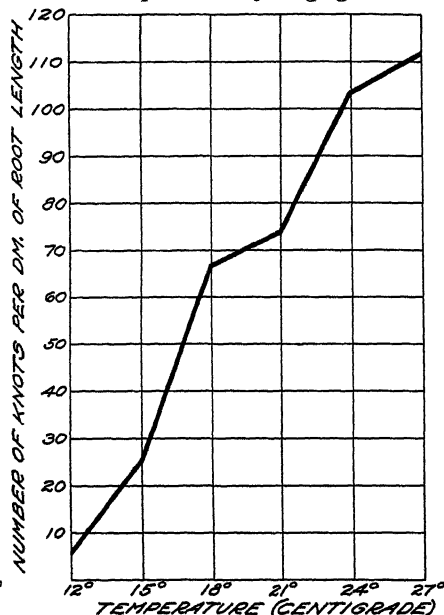


FIG. 17.—Effect of temperature on development of root knot in potatoes

The combined effects produced by temperature on the different growth factors were strikingly evident in the growth of the plants. At 12°, 15°, and 18° C. the plants were well supplied with beneficial nodules and were practically free from nematode root knots, and splendid growth occurred. Growth was greatly reduced at 24° and 27°, where root knot was abundant and beneficial nodules lacking. Figure 9, B, shows the root growth in the 12° tank, with good nodules and entire freedom from root knot. The plants grown at 15° were practically the same. Figure 9, C, shows the root of a plant grown at 27° C., with no nodules and abundance of root-knot infection.

With the potatoes the abundance of heavy root growth and the obvious uniformity of infection made it seem unnecessary to make complete counts of numbers of knots. Consequently, typical roots were selected from each plant and measured and number of knots counted. Estimates for the entire plant in number of knots per unit of length of root were made on this basis. The results as shown in the graph in Figure 17 agree with all previous results with other plants, in showing increase in infection with rise in temperature. This rise was particularly uniform with the potato.

TABLE 7.—Effect of temperature on root-knot development in vetch

Temperature and can No.	Length	Beneficial nodules	Knots	Knots per d. m.	Mean
12° C.:	<i>Cm.</i>		<i>Number</i>	<i>Number</i>	
Can 1.....	180	Clustered on upper part of main root and scattered throughout.	0		
Can 2.....	158	Scattered throughout root.	0		
Can 3.....	168	One cluster on main root.	0		
Can 4.....	143	3 small clusters.	0		
Can 5.....	148	2 clusters.	0		
15° C.:					
Can 1.....	133	Clustered on main root and scattered throughout.	17	1.278	
Can 2.....	136	Scattered.	29	2.132	
Can 3.....	98	Scattered throughout.	9	.918	
Can 4.....	152	A few scattered.	30	1.973	
Can 5.....	146	Cluster on main root.	29	1.986	1.657±.143
18° C.:					
Can 1.....	148	Few and scattered.	42	2.84	
Can 2.....	93	Few.	51	5.48	
Can 3.....	170	do.	53	3.12	
Can 4.....	182	Cluster on main root.	67	3.68	3.78±.31
Can 5.....					
21° C.:					
Can 1.....	107	Few.	84	7.85	
Can 2.....	143	do.	106	7.16	
Can 3.....					
Can 4.....	113	Different species, few nodules. Angustifolia.	167	14.78	
Can 5.....	52	None.	44	8.46	9.56±.92
24° C.:					
Can 1.....	132	Few.	114	8.64	
Can 2.....	40	Very few.	52	13.00	
Can 3.....	145	Few, scattered.	132	9.10	
Can 4.....	78	Very few.	169	21.66	
Can 5.....	37	None.	132	37.67	18.01±2.13
27° C.:					
Can 1.....	50	Two.	110	22.00	
Can 2.....	67		155	23.13	
Can 3.....	28	Clustered about main root.			
Can 4.....	41	Very few.	83	20.24	
Can 5.....	87	do.	116	13.33	19.68±1.25

TABLE 8.—Effects of temperature on root-knot development in potato

Temperature and can No.	Number of roots	Specimen root examined			
		Length	Number of knots	Number of knots per dm.	Mean
12° C.:		<i>Cm.</i>			
Can 1.....	23	15	6	4.000	
Can 2.....	22	9	4	4.444	
Can 3.....	17	9	7	7.777	5.407±0.66
15° C.:					
Can 1.....	19	15	27	18.00	
Can 2.....	14	15	54	36.00	
Can 3.....	15	16	34	21.25	25.08±13.05
18° C.:					
Can 1.....	14	9	58	64.44	
Can 2.....	10	14	105	75.00	
Can 3.....	23	10	60	60.00	66.48±2.45
21° C.:					
Can 1.....	15	16	108	67.50	
Can 2.....	14	14	110	78.57	
Can 3.....	22	9.5	70	73.68	73.25±1.76
24° C.:					
Can 1.....	19	15	147	98.00	
Can 2.....	11	7	95	135.71	
Can 3.....	15	10	75	75.00	102.90±9.74
27° C.:					
Can 1.....	15	14	168	120.00	
Can 2.....	14	13	155	119.23	
Can 3.....	9	11	105	95.45	111.56±4.54

EFFECT OF TEMPERATURE ON THE PATHOLOGICAL ANATOMY OF THE HOST

Frequent observations during the recording of results of temperature-control experiments indicated marked differences in type of knot formed at the higher and the lower temperatures. This was best shown in the case of tobacco in experiment 4. At 25° C. the knots were not only more abundant than at 19°, but they were smaller, asymmetrical in shape, different in color, and indicated a difference in the relation of the parasite to the host plant. Close examination revealed that the nematodes were more frequently mature in the 25° tanks, and that dark masses on the sides of the galls were due to the extruded egg masses of the females. This stage in their maturity was reached before the galls had developed to the size of those in the 19° tank. The smaller, more mature galls were more injurious to the host than the others, for the difference between controls and inoculated plants was always greater at the higher than at the lower temperatures.

The same type of differences occurred with other crops as well, notably with celery and cucumber.

In experiment 9 with the potato, a peculiar warty formation in the vascular region of the cut surface of the seed piece, at the higher temperatures, was found to be due to infection of the seed piece. Mature female nematodes were found within such galls. This was observed in several different cases. The seed pieces had previously been freshly cut on one surface, the other side having become protected by a corky layer, which had developed as the seed piece lay in moss for sprouting. Only the freshly cut surface was found infested in the manner just described. In ordinary practice, potato seed-piece infection is not likely to be much of a factor in nematode increase, in view of the fact that at the time the potatoes are planted the temperature of the soil is ordinarily too low for much activity on the part of the eelworms.

CONCLUSIONS

General observations pointing toward climate as one of the limiting factors in the distribution of the root-knot nematode have been justified by a few definite experiments.

The results of the soil-temperature experiments are evident and conclusive. Below about 16° C. the amount of root knot is substantially less than it is only two or three degrees higher. Three degrees lower yet it is almost eliminated and is practically negligible. At 10° and 12° infections are very rare. Different crops vary to some extent in amount of infection at points near the critical temperature. Tobacco shows the change very abruptly, from the lower to a higher temperature, whereas with soy bean the change appears to be more gradual. With both, the critical temperature is much below the optimum for growth. With crops whose range of good growth includes the critical temperature, the difference in root-knot development are striking. At the higher temperatures root knot is abundant up to as high as the plants will grow. There appeared at times to be a dropping off of amount of the disease above 30°, but this was usually traceable to a decrease in available root growth, rather than an actual decrease in proportion of affected roots.

The similarity in effect on all the plants studied, high-temperature-loving and low-temperature-loving plants alike, lead to the belief that the reaction to temperature is on the part of the parasite rather than the host. Differences in type of knots are produced at different temperatures, to be sure, but here again at least a part of the difference is due to differences in stage of development of the nematode.

There appears to be opportunity for economic application of the results of investigations described in this paper. Many successful commercial greenhouse men throughout the country, as well as truck growers in the South, make use of the fact of the warmth-requiring proclivities of the root-knot nematode to grow successfully low-temperature-enduring plants, such as lettuce and celery, in root-knot-infested ground. Stone and Smith (?) reported that they had grown lettuce in soil following badly diseased cucumbers, and that root knot did not appear on it. They attributed this to resistance of the lettuce to nematode infection, and could not account for the fact that they had elsewhere seen lettuce badly knotted, unless by the possible occurrence of different strains of nematodes. It is probable that their lettuce was grown at the temperature most favorable to it, which is below the critical point for severe nematode infestation. Greenhouse managers may now be able to adjust temperatures with root-knot control in mind, with a possible saving in costs. Similarly, southern truck growers may be able to so adjust time of planting celery, lettuce, and other low-temperature-loving plants, as to avoid to a greater extent losses due to root knot.

The results of the experiment with vetch would seem to add greatly to the argument for the use of winter vetches as southern soil-improvement crops, in some sections at least. They should be valuable in root-knot control rotations. Even if susceptible to root knot, their principal period of growth would be below critical temperature for root-knot infection. As far south as Brooksville, Fla., soil temperature records show that for the winter of 1920-21 the soil temperature at 12 inches in depth was below the critical temperature for infection for only a short period, approximately a week in January. Prior to that it was below for parts of a day only. The use of a susceptible vetch in this locality, therefore, would not be practicable.

Farther north, however, and yet within the region of serious root-knot damage, lower soil temperatures are attained. At Monetta, S. C., the records for a 5-inch depth show an almost constant temperature below 13° C. from about the 1st of December, 1921, to about the middle of April, 1922. At 12 inches it was approximately the same, with less fluctuation and a slower response to spring warmth. At this place, therefore, a hardy fall-planted vetch grows readily until well along in the spring, without risk of increasing the nematodes, and with definite soil improvement.

The amount of moisture seems to play only a small part in root-knot development, so long as the moisture content of the soil is favorable to the growth of the crops. Within the range of 40 to 80 per cent of the moisture-holding capacity of the soil there is very little difference in root-knot development. At 60, 70, and 80 per cent there appears to be a slight increase over the other percentages. Even below 40 per cent, which is too dry for good growth of ordinary crops, and above 80, which is more or less muddy, considerable root knot occurs.

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A STUDY OF SOIL HETEROGENEITY IN EXPERIMENT PLOTS¹

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INTRODUCTION

In carrying on plot experiments the agronomist is continually confronted with the problem of soil heterogeneity, a problem so important that no field experiments are carefully planned without considering it. Several ways of overcoming the effect of soil heterogeneity have been suggested, one of which is replication of plots in such manner as to take into account the uniformity of the soil as revealed by production.

A few years ago the West Virginia Agricultural Experiment Station acquired about 16 acres of second-bottom land in Mason County near the Ohio River at Maggie, W. Va. The tract is level and to the eye appears quite uniform. It is mapped as "Wheeling fine sandy loam" by Bureau of Soils² of the United States Department of Agriculture. The greater part of the tract was available for rotation experiments, but before beginning the experiments it was decided to make a soil-uniformity test by growing blanket crops for two successive years.

PLAN OF THE FIELD

In all, 270 plots, each 68 feet by 21 feet, were laid off for the rotation experiments. Although the gross area of a plot was approximately $\frac{2}{3}$ of an acre, yield was based on an area 61 feet by 14 feet only. A border $3\frac{1}{2}$ feet wide on the sides and ends of each plot was discarded at harvest, the area upon which yield was based being approximately $\frac{1}{3}$ of an acre. The general plan of the field experiment is shown in Figure 1.

The plots were laid out in three double series, with a 14-foot roadway around the entire field and between the double series. The plot numbers, and the plots used for a certain rotation, are given in the diagram, the latter being indicated by letter. The letter a is found in plots 1, 13, 25, and 33, and a' is found in plots 111, 123, 135, and 143. The crop rotation for the first series of plots is corn first year, wheat second year, clover and timothy third year, and potatoes fourth year, with a cover crop between certain crops. The number of plots and the number of years to complete the rotation were arranged to correspond, so that each crop would be grown each year. This cropping system is duplicated in plots 111, 123, 135, and 143, designated by a'. For example, in 1925 plots 1 and 111 were occupied by corn, 13 and 123 by wheat, 25 and 135 by clover and timothy,

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² LATIMER, W. J., and MOONEY, C. N. SOIL SURVEY OF THE POINT PLEASANT AREA, WEST VIRGINIA. U. S. Dept. Agr., Bur. Soils, Field Oper. 1910, Rpt. 14: 1077-1122, illus. 1912.

and 33 and 143 by potatoes. The scheme of replication for the other rotations is indicated in similar manner—a certain letter indicates a given rotation, and the same letter primed indicates the duplicate plots.

Plot	Plot		Plot	Plot		Plot	Plot
Roadway							
1 a	51		101 s	151		201 m'	251
2 b	52		102 t	152		202 n'	252
3 c	53		103 q	153		203 o'	253
4 d	54		104 v	154		204 m'	254
5 e	55		105 r	155		205 n'	255
6 f	56		106 q	156		206 o'	256
7 g	57		107 q	157		207 m'	257
8 h	58		108 u	158		208 n'	258
9 i	59		109 v	159		209 m'	259
10 j	60		110 x	160		210 w'	260
11 k	61		111 a'	161		211 p	261
12 l	62		112 b'	162		212 p	262
13 a	63		113 c'	163		213 p	263
14 b	64		114 d'	164		214 p	264
15 c	65		115 e'	165		215 q	265
16 d	66		116 f'	166		216 r	266
17 e	67		117 g'	167		217 s	267
18 f	68		118 h'	168		218 t	268
19 g	69		119 i'	169		219 u	269
20 h	70		120 j'	170		220 q	270
21 i	71		121 k'	171		221 r	271
22 j	72		122 l'	172		222 s	272
23 k	73		123 a'	173		223 t	273
24 l	74		124 b'	174		224 q	274
25 a	75		125 c'	175		225 s'	275
26 b	76		126 d'	176		226 r	276
27 c	77		127 e'	177		227 t'	277
28 d	78		128 f'	178		228 q'	278
29 e	79		129 g'	179		229 u'	279
30 f	80		130 h'	180		230 r'	280
31 g	81		131 i'	181		231 q'	281
32 h	82		132 j'	182		232 q'	282
33 a	83		133 k'	183		233 q'	283
34 b	84		134 l'	184		234 r'	284
35 c	85		135 a'	185		235 s'	285
36 w	86		136 b'	186		236 t'	286
37 m	87		137 c'	187		237 u'	287
38 n	88		138 d'	188		238 q'	288
39 o	89		139 e'	189		239 r'	289
40 m	90		140 f'	190		240 v'	290
41 n	91		141 g'	191		241 t'	291
42 o	92		142 h'	192		242 q'	292
43 m	93		143 a'	193		243 r'	293
44 n	94		144 b'	194		244 s'	294
45 m	95		145 c'	195		245 v'	295
Roadway							

FIG. 1.—Field plan of the plots used in the rotation experiments at Maggie, W. Va.

In Figure 1 letters are given to only the first series of each double series of plots. The system of cropping in the second series was similar to the first series, and the manner of replication in the two series exactly corresponded. The difference between the first and second members of each double series is a matter of lime treatment, of cover crops, or of handling crop residues. The duplicates of plots 51, 63, 75, and 83 are 161, 173, 185, and 193, respectively. In similar manner the cropping system and the method of replication with respect to the other crop rotations in the second series follows that of the first series.

THE CROPS AND METHODS OF DETERMINING YIELDS

Before actually beginning the rotation experiments, and before any differential treatment was given to the plots, the whole area diagrammed in Figure 1 was cropped first (in 1923) to oats, and then (in 1924) to winter wheat. Fulghum oats and Gladden wheat were used. Both crops were seeded with a grain drill, and the rows were run lengthwise of the plots. The oats was seeded at the rate of approximately $2\frac{1}{2}$ bushels per acre, and the wheat at about 6 pecks per acre. The oats was harvested for hay and the wheat for grain. The crops on the borders of each plot were removed before harvest, and in the case of oats the hay produced on the entire net area (61 by 14 feet) of each plot was weighed. At the same time a sample of approximately 500 gm. of hay was taken and carefully weighed. These samples were hung in a well-ventilated shed, and when they reached a relatively constant weight they were weighed again. The second weighing was on a dry, sunshiny day. The amount of air-dry material produced per plot was computed from the data so obtained.

The yield of wheat was determined in the manner suggested by Arny and Garber.³ There were no facilities for threshing the grain of each plot separately, so the yields were determined from five rod rows from each plot. One drill row 16 feet long was removed from the corner of each plot (after borders had been removed), and one drill row of the same length was taken from the middle of the plot. No attempt was made to keep the rod rows of any one plot separate. This material in bundles was hung in a shed until dry and was then weighed. After the bundles were weighed the spikes were removed from the straw and shipped to Morgantown, where they were threshed and the yield of grain determined. The yield in bushels per acre of each plot was calculated from these results. The yield of the straw was obtained by subtracting the weight of the grain from the total weight of the rod-row bundles.

After the rod rows were removed each plot was harvested separately with a grain binder. The bundles were tagged and set up in small shocks and covered with canvas. After the shocks had thoroughly dried out under these conditions the bundles from each plot were weighed. In this way the yield of grain plus straw was determined.

YIELDS BASED ON ROD-ROW SAMPLES

Before discussing the plot yields as a measure of soil uniformity it may be well to present certain data obtained in 1924 in connection with determining the wheat yields. As has been stated, the yields of wheat in bushels per acre were based on five rod rows taken from each plot. The weight of the straw of the rod-row samples was determined by subtracting the weight of the threshed grain from the total weight. In addition to these data, the weight of the air-dry wheat bundles (straw plus grain) remaining on each plot after the rod rows were removed was determined. By adding this weight to the weight of the rod-row bundles the total production of grain and straw on each plot was ascertained. The interrelation of these

³ ARNY, A. C., and GARBER, R. J. FIELD TECHNIC IN DETERMINING YIELDS OF PLOTS OF GRAIN BY THE ROD-ROW METHOD. *Jour. Amer. Soc. Agron.* 11: 33-47, illus. 1919.

data, as well as the relation between these data and the computed yield of grain for the entire plot, was studied. By means of the ratio of grain to straw from the rod rows, and the total weight of the grain plus the straw from the entire plot, the yield of the grain on each entire plot was computed.

The correlation between the yield of grain and the yield of grain plus straw, all based on the rod-row samples, is shown in Table 1. The table shows a marked positive correlation ($+0.951 \pm 0.004$) between these two sets of variables. The regression equation for yield of grain on yield of grain plus straw is $Y_g = -0.505 + 5.478X_g + s$. The standard error of estimate, as determined by the usual formula ($\sigma_y \cdot x = \sigma_y \sqrt{1 - r^2}$), is 1.207, or about 30.9 per cent of the standard deviation of the yield of grain. These facts show that in this experiment one may predict with a fair degree of precision the yield of grain from the yield of grain plus straw.

TABLE 1.—Correlation of yield of grain from five rod rows with yield of grain plus straw from five rod rows at Maggie, W. Va.

	Grain plus straw, pounds															
	1.5	1.8	2.1	2.4	2.7	3	3.3	3.6	3.9	4.2	4.5	4.8	5.1	5.4	5.7	
Grain, bushels per acre:																
7.5	1	1														2
9		4	3	0												7
10.5		3	16	4	1											24
12			10	21	3											34
13.5				13	17	1										31
15				1	31	19										51
16.5						25	17	1								43
18					1	4	22	7								34
19.5				1	1		2	8	3							15
21								5	10	1		1				17
22.5									1	0						1
24									1	2						3
25.5											1	1				2
27													1	1		2
28.5													1			1
30													1	1	1	3
	1	8	29	40	54	49	41	21	15	3	1	2	3	2	1	270

$$r = +0.951 \pm 0.004.$$

In view of the foregoing observations, and if the five rod rows from each plot are representative of the plot, a marked positive correlation would be expected between the yield of grain based on the rod-row samples and the yield of the total grain plus straw based on the entire plot. This expectation was fulfilled, as is shown in Table 2. Although the correlation coefficient (0.841 ± 0.012) obtained is not as high as the one just discussed above, it nevertheless shows a marked positive correlation between the two sets of variables. The regression equation for yield of total grain plus straw on yield of grain from the rod rows is $y_g + s = 13.147 + 2.269X_g$. The standard error of the estimated yields of grain plus straw by means of the regression equation is 5.699, or about 54.1 per cent of the standard deviation of the yields of grain plus straw. It is obvious that the predicted values from this equation are considerably less trustworthy than the predicted yields of grain from the yield of grain plus straw all based on the rod-row samples.

TABLE 2.—*Correlation of yield of grain plus straw from entire plot with yield of grain from five rod rows at Maggie, W. Va.*

	Grain bushels per acre (rod rows)																
	7.5	9	10.5	12	13.5	15	16.5	18	19.5	21	22.5	24	25.5	27	28.5	30	
Grain plus straw, pounds (entire plot):																	
22.5		1															1
27.5	2	2	2	1													7
32.5		2	7	2													17
37.5		1	10	12	4	3		1									30
42.5		1	3	11	14	15	2	2									50
47.5				6	9	18	17	9	2								63
52.5				1	3	13	12	12	4	3							48
57.5					1	1	6	9	5	5							28
62.5						1	4	2	2	5	1	1					16
67.5							1			3		1	1				6
72.5												1	1				2
77.5														1			1
82.5										1				1	1	2	5
87.5															1	1	1
	2	7	24	34	31	51	43	34	15	17	1	3	2	2	1	3	270

$$r = +0.841 \pm 0.012$$

In addition to the two coefficients of correlation mentioned certain others were calculated. The correlation coefficient obtained for the yield of grain plus straw from the rod rows and the yield of grain plus straw from the entire plot was $+0.872 \pm 0.010$; for the yield of grain from the rod rows and the computed yield of grain from the entire plot, $+0.894 \pm 0.008$; for the computed yield of grain from the entire plot and the yield of grain plus straw from the entire plot, $+0.940 \pm 0.005$; and for the yield of grain plus straw from the rod rows and the computed yield of grain from the entire plots, $+0.844 \pm 0.012$. In all cases the coefficients show a marked positive correlation. Of particular interest is the high correlation between the yield of the grain plus straw from the rod rows and the total yield of the grain plus straw from the entire plot. This high correlation shows that the weight of the rod-row samples (grain plus straw) from each plot is a fairly reliable index to the total weight of the grain plus straw produced on that plot. The high positive correlations obtained between the computed yields of the entire plots and the experimentally determined yields of the plots are expected, in view of the high correlations found among the latter.

It is believed that the facts brought out above justify the use of rod-row samples in determining yield. The discussion of plot uniformity which follows later is based on the yield of wheat in bushels per acre as determined from the rod-row samples removed from each plot.

PLOT UNIFORMITY AS SHOWN BY YIELDS OF OAT HAY

* The yield of air-dry oat hay in pounds per acre is shown in Table 3. The mean yield of all the plots is 1,883.7 pounds. It will be observed that the yield of each plot is expressed in the table as a deviation from this mean yield. If the yield of a particular plot is less than the mean yield it is indicated as a minus deviation; on the other hand, if the yield of a plot is greater than the mean yield it is shown as a plus deviation.

TABLE 3.—Yield of oat hay in pounds of air-dry material per acre per plot, expressed as deviations from the mean yield of all the plots in the crop-rotation experiments at Maggie, W. Va. Yield based on the production of the net area of each plot in 1923

Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot
1	+39.3	+182.3	51	101	+59.3	+258.3	151	201	+39.3	+80.3	251
2	-37.7	+59.3	52	102	+39.3	+171.3	152	202	-123.7	+136.3	252
3	+23.3	+212.3	53	103	-67.7	-32.7	153	203	-1.7	+243.3	253
4	-52.7	+161.3	54	104	-103.7	+80.3	154	204	-154.7	+406.3	254
5	-32.7	+493.3	55	105	+666.3	+717.3	155	205	+345.3	+758.3	255
6	+34.3	+253.3	56	106	+880.3	+952.3	156	206	+273.3	+431.3	256
7	+207.3	+426.3	57	107	+896.3	+839.3	157	207	+304.3	+105.3	257
8	+324.3	+294.3	58	108	+493.3	+391.3	158	208	+396.3	+74.3	258
9	+29.3	+18.3	59	109	+340.3	+350.3	159	209	+187.3	+29.3	259
10	+156.3	+248.3	60	110	+176.3	+23.3	160	210	+309.3	+360.3	260
11	-333.7	-67.7	61	111	+278.3	-174.7	161	211	-72.7	+1,523.3	261
12	-108.7	-78.7	62	112	-62.7	-108.7	162	212	+64.3	+2,650.3	262
13	+5.3	-123.7	63	113	-256.7	-149.7	163	213	-16.7	+2,655.3	263
14	-42.7	+29.3	64	114	-241.7	-164.7	164	214	-16.7	+1,100.3	264
15	+238.3	-1.7	65	115	-445.7	-246.7	165	215	-123.7	+156.3	265
16	+136.3	+299.3	66	116	-220.7	-108.7	166	216	-159.7	+23.3	266
17	+8.3	-185.7	67	117	-480.7	-83.7	167	217	-62.7	-88.7	267
18	-232.7	-190.7	68	118	-169.7	-419.7	168	218	-231.7	-144.7	268
19	-246.7	-88.7	69	119	-266.7	-88.7	169	219	-220.7	-78.7	269
20	-582.7	-215.7	70	120	-266.7	-180.7	170	220	-169.7	+105.3	270
21	-266.7	-195.7	71	121	-195.7	-180.7	171	221	-139.7	-1.7	271
22	-419.7	-164.7	72	122	-93.7	-1.7	172	222	+59.7	-16.7	272
23	+8.3	+49.3	73	123	-210.7	-180.7	173	223	-134.7	+23.3	273
24	-266.7	-174.7	74	124	-287.7	-266.7	174	224	-378.7	-103.7	274
25	-307.7	-322.7	75	125	-292.7	+3.3	175	225	-282.7	-134.7	275
26	+13.3	-338.7	76	126	-256.7	-246.7	176	226	-261.7	-327.7	276
27	-292.7	-256.7	77	127	-67.7	-159.7	177	227	-231.7	-246.7	277
28	-363.7	-317.7	78	128	-251.7	-210.7	178	228	-220.7	-338.7	278
29	-282.7	-424.7	79	129	-307.7	-190.7	179	229	-419.7	-470.7	279
30	-215.7	+23.3	80	130	-88.7	-154.7	180	230	-210.7	-205.7	280
31	+748.3	+59.3	81	131	+238.3	-200.7	181	231	-210.7	-47.7	281
32	+549.3	+340.3	82	132	+13.3	-83.7	182	232	-139.7	-72.7	282
33	+656.3	+166.3	83	133	-134.7	-180.7	183	233	-129.7	-47.7	283
34	-93.7	-149.7	84	134	-271.7	-62.7	184	234	-190.7	+248.3	284
35	+156.3	+105.3	85	135	-220.7	-333.7	185	235	-348.7	-83.7	285
36	+258.3	+258.3	86	136	-67.7	-1.7	186	236	-271.7	-118.7	286
37	+131.3	+253.3	87	137	+115.3	+125.3	187	237	-266.7	+29.3	287
38	+100.3	+182.3	88	138	+396.3	+202.3	188	238	-465.7	-358.7	288
39	+80.3	+156.3	89	139	+314.3	+574.3	189	239	-225.7	-195.7	289
40	+141.3	+23.3	90	140	+488.3	+34.3	190	240	-741.7	-317.7	290
41	+324.3	+340.3	91	141	+564.3	-88.7	191	241	-674.7	-598.7	291
42	+131.3	-353.7	92	142	-88.7	-169.7	192	242	-368.7	-307.7	292
43	+49.3	-251.7	93	143	-802.7	-72.7	193	243	-669.7	-169.7	293
44	-11.7	-159.7	94	144	-572.7	+207.3	194	244	-180.7	+64.3	294
45	-185.7	-205.7	95	145	-287.7	+64.3	195	245	+187.3	-190.7	295

Mean=1,833.7 pounds.

The correlation of yields between contiguous plots, as suggested by Harris,⁴ was calculated, the magnitude of the correlation so obtained indicating the degree of the soil heterogeneity of this field. If the field is "patchy," the yields of contiguous plots will tend to be of the same order and therefore a high correlation will be obtained.

In applying the formula⁵ suggested by Harris the plots were first grouped in a 2×2-fold manner.

⁴ HARRIS, J. A. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. Amer. Nat. 49: 430-454, illus. 1915.

(5)
$$r_{p_1 p_2} = \frac{\{[S(C^2 p - S(p^2))/m[n(n-1)]]\} - \bar{p}^2}{\sigma p^2}$$

when S indicates summation, p the yield of the ultimate plots, Cp the yield of the combination plots grouped in a certain manner, n the number of ultimate plots in each combination plot, m the number of combination plots, \bar{p} the average yield of the ultimate plots, and σp their standard deviation.

For example, plots 1, 2, 51, and 52 were grouped together, and 3, 4, 53, and 54 were grouped together, and the other plots were grouped in a similar way, with the following exceptions: Plots 45, 95, 245, and 295 were placed in one group and 109, 159, 111, and 161 in another group. The first exception was made to take care of the last pair of plots in the first and third double series, respectively. The reason for the other exception is explained below.

Plots 211 to 214, and 261 to 264, inclusive, were eliminated from this study because of the fact that a few years ago a straw stack had stood on or in the vicinity of plot 263, which undoubtedly accounts for the relatively high yields on plots 261 to 264, inclusive. Plots 110 and 160 were eliminated because in the rotation experiments they were to be used for a continuous cropping study and were not to be replicated.

The plots were also grouped in a 3×2 -fold manner—for example, plots 1, 2, 3 were grouped together, and plots 51, 52, and 53 were grouped together, etc.—and the coefficients of correlation were calculated. The irregularity in the 3×2 -fold grouping occurred with plots 144, 145, 194, 195, 245, and 295, which were grouped together. In addition to the plots already mentioned, plots 210 and 260 also were eliminated from consideration in the 3×2 -fold groups.

After calculating the two coefficients of correlations for yields of contiguous plots in the manner mentioned above, a third correlation coefficient was calculated by means of the formula suggested by Harris for the yields of replicated plots. The plots were placed in groups of fours made up according to the replications which it was proposed to use in the rotation experiments. (See fig. 1.) For example, plots 1, 51, 111, and 161 were placed in one group, plots 2, 52, 112, and 162 were placed in another group, and similarly each pair of plots (end to end) was grouped with its proposed replicate in the rotation experiments.

In addition to testing for soil uniformity by Harris's method, one may obtain a measure of uniformity by correlating in the usual way the yields of contiguous plots. This was done for the yields of the plots under consideration. First a correlation table was prepared correlating the yields of plot 1 with 2, 2 with 3, 3 with 4, and proceeding in this way to the end of the series (plot 45) and then beginning at plot 51 and correlating it with 52, 52 with 53, etc. In this manner a correlation table was prepared which included all the plots except those eliminated, namely, 110, 160, 211, 212, 213, 214, 261, 262, 263, and 264. Where the continuity of a plot series was broken on account of the elimination of certain plots, the plots immediately above and below the "break" were treated the same as the last and first plot, respectively, of a series. In a somewhat similar manner a correlation table was prepared, using the yield of each plot but once. For example, the yield of plot 1 was correlated with the yield of plot 2, plot 3 with 4, plot 5 with 6, etc., proceeding in a regular manner from top to bottom of each series, except in the cases where plots were eliminated. To distinguish the two correlation coefficients so obtained, the first is designated as "all contiguous plots" and the second as "paired contiguous plots."

To further test the effectiveness of the proposed manner of replication in overcoming soil heterogeneity, still another correlation table was prepared in which the yield of each plot was correlated

with the yield of its replicate. For example, the yield of plot 1 was correlated with the yield of plot 111, 51 with 161, 2 with 112, 52 with 162, and so on for the rest of the plots in the rotation experiments. The correlation coefficient so obtained is designated as "paired replicated plots."

The correlation coefficients obtained by Harris's method, together with those calculated in the usual manner, are given in Table 4. The coefficients obtained by the two methods agree, in that they show a rather marked soil heterogeneity for the experimental field under observation. Also, the particular manner of replication was helpful in overcoming the objectionable effect of soil heterogeneity.

TABLE 4.—Coefficients of correlation between yields of oat hay grown on certain plots at Maggie, W. Va., in 1923

Method of calculation	<i>n</i>	<i>r</i>
Harris's 2×2-fold contiguous	260	+0.631±0.025
Harris's 2×3-fold contiguous	258	+0.542±0.030
Harris's 2×2-fold replicated	260	+0.134±0.041
Ordinary "all contiguous plots"	250	+0.668±0.024
Ordinary "paired contiguous plots"	130	+0.694±0.031
Ordinary "paired replicated plots"	130	−0.068±0.059

The correlation coefficient obtained by applying Harris's formula to the yields of contiguous plots, are, for the 2×2-fold grouping $+0.631 \pm 0.025$, and for the 2×3-fold grouping $+0.542 \pm 0.030$; the difference between the coefficients is 0.089 ± 0.039 , a difference which is only slightly more than two times its probable error. (All probable errors of differences were calculated by the approximate formula $D_e = \sqrt{e^2_1 + e^2_2}$.) When Harris's formula is applied to replicated plots grouped in a 2×2-fold manner, a correlation coefficient of $+0.134 \pm 0.041$ is obtained which is significantly less than either of the coefficients obtained by grouping contiguous plots. This constant is approximately 3.3 times its probable error, and shows that there is only a slight correlation between the yields of replicated plots.

The correlation coefficient obtained by the ordinary method for "all contiguous plots" is $+0.668 \pm 0.024$, and for "paired contiguous plots" $+0.694 \pm 0.031$. These coefficients do not differ significantly from one another, and neither does either of them differ significantly from the coefficient obtained by Harris's formula applied to the yields of contiguous plots grouped in a 2×2-fold manner. The correlation coefficient obtained by the ordinary method for "paired replicated plots" is -0.068 ± 0.059 . The difference between this coefficient and the one obtained for replicated plots by Harris's method is 0.202 ± 0.072 , a difference which although it is considerable, is slightly less than three times its probable error.

PLOT UNIFORMITY AS SHOWN BY YIELDS OF WHEAT

The yields of wheat in bushels per acre based on the five rod rows removed from each plot are given in Table 5. As in the case of oat hay, the yields of wheat are expressed as deviations from the mean yield of all the plots. The yield of any particular plot is easily ascertained by adding or subtracting (as the case may be) the deviation from the mean yield (15.6 bushels).

TABLE 5.—Yield of wheat in bushels per acre per plot, expressed as deviations from the mean yield of all the plots in the crop-rotation experiments at Maggie, W. Va. Yield based on the average production of five rod rows removed from each plot at harvest in 1924

Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot
1	-0.5	+1.4	51	101	+2.9	-1.0	151	201	-5.2	-4.0	251
2	-6.0	+1.7	52	102	+0.2	-6.0	152	202	-6.6	-4.7	252
3	-0.6	-0.3	53	103	-4.1	-5.0	153	203	-6.7	-4.6	253
4	-3.1	-2.4	54	104	-7.0	-5.3	154	204	-7.8	-1.2	254
5	-1.3	-5.0	55	105	-1.3	-2.5	155	205	-7.7	-2.0	255
6	+1.7	-3.8	56	106	-1.4	+0.4	156	206	-3.9	-4.1	256
7	-0.1	+1.8	57	107	+6.4	-0.5	157	207	-5.6	-4.2	257
8	-2.2	+1.6	58	108	-2.0	-3.3	158	208	-1.2	-3.4	258
9	+3.5	+3.5	59	109	-2.9	-5.2	159	209	-4.1	-3.0	259
10	+3.5	+2.7	60	110	-5.8	-4.0	160	210	-4.3	-0.9	260
11	-4.9	-2.1	61	111	-0.6	-3.3	161	211	-4.7	+5.0	261
12	+0.7	-3.7	62	112	-2.4	-0.8	162	212	-1.3	+14.1	262
13	+0.9	-1.4	63	113	-1.2	+0.1	163	213	+0.7	+15.0	263
14	+1.0	+0.4	64	114	-5.3	-1.6	164	214	+2.0	+5.7	264
15	+4.0	+3.1	65	115	-0.8	+1.0	165	215	+0.1	+1.1	265
16	+2.8	+2.3	66	116	-1.6	-1.3	166	216	-3.6	-0.7	266
17	+0.4	+0.2	67	117	+2.2	-0.6	167	217	-1.0	+1.7	267
18	+0.6	-1.0	68	118	-0.3	-3.4	168	218	+0.7	-1.2	268
19	-4.8	-2.3	69	119	+1.8	+4.9	169	219	+0.5	-0.7	269
20	-4.7	-4.9	70	120	+1.2	-0.9	170	220	-2.7	+1.9	270
21	-5.2	-4.4	71	121	+2.7	+4.7	171	221	-0.3	+3.0	271
22	-2.1	-3.9	72	122	-1.9	+4.0	172	222	+3.6	+2.1	272
23	+2.6	-5.4	73	123	-0.8	-0.1	173	223	-2.9	-0.9	273
24	-3.5	-5.9	74	124	+5.2	+3.7	174	224	+4.8	+2.3	274
25	-2.0	-4.2	75	125	-1.0	+2.6	175	225	-1.0	+5.5	275
26	-0.8	+0.5	76	126	+3.5	+2.2	176	226	+3.3	+2.4	276
27	+1.9	-1.9	77	127	-1.0	+0.6	177	227	+1.0	-2.7	277
28	+1.2	+1.5	78	128	+0.1	-5.9	178	228	-1.7	-0.5	278
29	+4.1	-2.9	79	129	+0.8	-2.2	179	229	-1.9	-4.0	279
30	+1.7	-3.8	80	130	-1.2	+2.4	180	230	-4.9	+1.3	280
31	+11.1	+3.0	81	131	+1.7	+0.5	181	231	-1.7	+0.2	281
32	+10.9	+5.4	82	132	+2.9	+1.6	182	232	-3.0	-0.4	282
33	+10.5	+1.6	83	133	-0.7	+1.0	183	233	+2.5	+1.3	283
34	+6.0	+5.5	84	134	+0.9	+2.4	184	234	+0.5	+0.1	284
35	+5.2	+4.2	85	135	+1.4	+0.9	185	235	-1.2	+5.1	285
36	+1.6	+4.7	86	136	+6.1	+4.1	186	236	+4.1	+5.3	286
37	-2.4	+0.3	87	137	+2.8	+0.9	187	237	-2.4	-0.7	287
38	-5.7	+0.9	88	138	+8.4	+15.0	188	238	-2.3	-0.2	288
39	-0.9	+1.9	89	139	+4.7	+12.6	189	239	+0.9	+0.3	289
40	-0.2	-1.4	90	140	+8.8	+7.9	190	240	-1.3	+3.2	290
41	+0.5	-5.1	91	141	+5.3	+1.9	191	241	-2.7	-3.3	291
42	-0.8	-0.3	92	142	+1.9	+0.9	192	242	-1.4	+4.3	292
43	-0.7	-3.7	93	143	-4.5	+1.6	193	243	-3.2	-3.6	293
44	-4.0	-2.1	94	144	-5.1	-3.6	194	244	+0.4	-0.4	294
45	-0.4	-5.1	95	145	-3.0	-1.7	195	245	-2.9	-2.8	295

Mean=15.6 bushels.

Correlation coefficients were calculated for the yields of wheat in exactly the same manner as for oat hay. The data are presented in Table 6.

TABLE 6.—Coefficients of correlation between yields of wheat grown on certain plots at Maggie, W. Va., in 1924

Method of calculation	n	r
Harris's 2X2-fold contiguous	260	+0.565±0.028
Harris's 2X3-fold contiguous	258	+0.516±0.031
Harris's 2X2-fold replicated	260	+0.248±0.039
Ordinary "all contiguous plots"	250	+0.553±0.030
Ordinary "paired contiguous plots"	130	+0.617±0.037
Ordinary "paired replicated plots"	130	+0.037±0.059

As in the case of the yields of oat hay, the coefficients of correlations based on the yields of wheat grain show that the experimental field was heterogeneous, and that the particular manner of plot replication was helpful in overcoming the objectionable effect of soil heterogeneity.

The correlation coefficients in Table 6 obtained by applying Harris's formula to the yields of contiguous plots are, for the 2×2 -fold grouping, $+0.565 \pm 0.028$; and, for the 2×3 -fold grouping, $+0.516 \pm 0.031$. The difference between the two coefficients is small and not significant. When the same formula is applied to replicated plots grouped in a 2×2 -fold manner, the coefficient of correlation obtained is $+0.246 \pm 0.039$, or about one-half the magnitude of the coefficients based on contiguous plots. This coefficient is approximately six times its probable error and therefore statistically significant. However, the coefficient is low and shows that the correlation between the yields of replicated plots is not marked.

The correlation coefficient obtained by the ordinary method for "all contiguous plots" is $+0.553 \pm 0.030$, that for the "paired contiguous plots" is $+0.617 \pm 0.037$, and that for "paired replicated plots" $+0.037 \pm 0.059$. Here, as in the case of yields of oat hay, the correlation between the wheat yields of replicated plots is sensibly zero. The difference between the coefficients of yields of replicated plots based on the two methods of calculation is 0.209 ± 0.071 , a difference slightly less than three times its probable error. In both the yields of oat hay and of wheat grain the correlation coefficients obtained by the ordinary method of calculation show a somewhat greater difference between contiguous and replicated plots than do the coefficients calculated by Harris's formula.

REGRESSION OF YIELDS IN CONTIGUOUS AND REPLICATED PLOTS

To obtain a graphic representation of the relationship between the yields in contiguous as compared with the yields in replicated plots, certain regression equations were calculated, and by means of these the regression lines were drawn. The data showing the correlation by the ordinary method of calculation between the yields of contiguous plots and between the yields of replicated plots for both the oat hay and wheat grain were used.

Before calculating the regression equations, appropriate tests were made to determine linearity. For this purpose the formula⁶ suggested by Blakeman⁷ for testing the significance of the difference between η^2 and r^2 was used. The correlation coefficients for "paired contiguous plots" and "paired replicated plots" are given in Table 4 for the yields of oat hay and in Table 6 for the yields of wheat. The correlation ratios for the same sets of paired variables are given in Table 7.

⁶ *P. E. diff.* = $2 \times 0.6745 / \sqrt{n} \sqrt{\eta^2 - r^2} \sqrt{(1 - \eta^2)^2 - (1 - r^2)^2} + 1$.

⁷ BLAKEMAN, J. ON TESTS FOR LINEARITY OF REGRESSION IN FREQUENCY DISTRIBUTIONS. *Biometrika* 4: 332-350. 1905.

TABLE 7.—Correlation ratios and the significance of the differences between the η^2 and r^2 values for the yields of "paired contiguous plots" and the yields of "paired replicated plots" at Maggie, W. Va.

	Crop	n	η^{yz}	Diff./ η^2 & r^2 /P.E.
"Paired contiguous plots".....	Oat hay...	130	0.729±0.028	1.9
"Paired replicated plots".....	do.....	130	.373±.051	3.6
"Paired contiguous plots".....	Wheat.....	130	.654±.034	1.9
"Paired replicated plots".....	do.....	130	.206±.057	1.8

The correlation ratios based on the yields of oat hay are 0.729 ± 0.028 for the "paired contiguous plots" and 0.373 ± 0.051 for the "paired replicated plots"; whereas the correlation ratios based on the yields of wheat grain are 0.654 ± 0.034 for the "paired contiguous plots" and 0.206 ± 0.057 for the "paired replicated plots." In all cases except one, the difference between η^2 and r^2 is not significant, as may be seen from the last column of Table 7. The exception is in the case of the "paired replicated plots" of oat hay. The question naturally arises whether the correlation ratio obtained here is of significance, or is due to accidental fluctuations. It is well known that relatively low values of η are quite likely to be too high, and consequently for paired variables which show little or no correlation the statistical value of η is questionable.

In Table 8 are the data from which the r and η values for the yields of oat hay in the "paired replicated plots" were calculated. This table shows several paired variables which are considerably removed from the main field of the distribution. It is possible that some of these outlying variables are accidental fluctuations and, as such, increase the magnitude of the correlation ratio.

TABLE 8.—Correlation between yields of oat hay produced in 1923 on replicated plots of the rotation series at Maggie, W. Va.

	Deviation in pounds per acre of air-dry hay															
	-850	-750	-650	-550	-450	-350	-250	-150	-50	+50	+150	+250	+350	+450	+550	+750
Deviation in pounds per acre of air-dry hay:																
-550.....									1*							1
-450.....										1						2
-350.....						2	1	2	1		1	1	1	1		9
-250.....			1			1	4	1		1	3	1	1			14
-150.....		1			1	1	3	5	3	1	1	1		1		18
-50.....			1	1	1	2	3	3	3	3	1		1			17
+50.....						2	9	4	4	2				1		23
+150.....						1	4	4	2	2	1	2				16
+250.....					2		2	4	2				2			11
+350.....					1			3		1	1		1		1*	7
+450.....							1		1							3
+550.....									1							1
+750.....	1*						1									2
+850.....							1								1	2
+950.....							1	1	1*							3
+1050.....																1
	1	1	2	2	6	9	30	27	18	11	3	7	6	3	3	130

$r = -0.068 \pm 0.059.$

In calculating the correlation ratio for the dependence of y on x in Table 8, it was found that a single paired variable contributed almost one-fourth of the total value obtained in the $N_x(\bar{y}_x - \bar{y})^2$ column. The value for $\sum N_x(\bar{y}_x - \bar{y})^2$ was 150.8398, whereas the value for $N_x(\bar{y}_x - \bar{y})^2$ of the first \bar{y} -array was 37.1161. Here is fairly good evidence that 1 of the 130 paired variables has contributed a disproportionate weight in determining the magnitude of the correlation ratio; and, moreover, it is quite possible that this outlying paired variable is accidental.

If the four outermost paired variables are eliminated, as is indicated by (e) in Table 8, the r and η values become -0.075 ± 0.060 and 0.331 ± 0.053 , respectively. The difference between their squares is 0.104 ± 0.035 , or 2.97 times its probable error. From this it follows

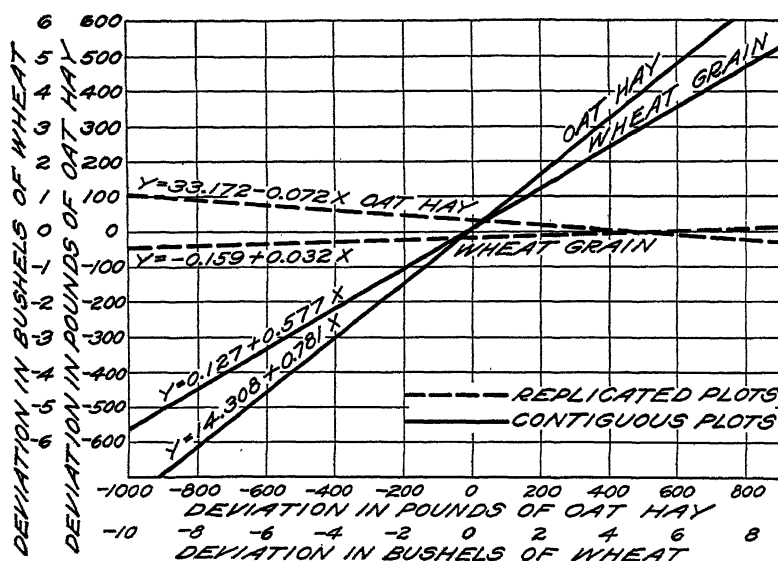


FIG. 2.—Regression lines for the yields of "paired contiguous plots" and of "paired replicated plots" in the rotation experiments at Maggie, W. Va.

that the odds are 1 to 22 that regression is linear. Although the odds in this case are rather high against the probability that regression is linear, it is certainly significant that by eliminating 4 of the 130 paired variables the odds are lowered from 65 to 1 to 22 to 1. In view of the above, a regression line for the yields of the "paired replicated plots" of oat hay was calculated and plotted, on the assumption that regression was linear.

The regression lines and their respective equations are shown in Figure 2. The effectiveness of replication in equalizing yield differences between plots due to soil heterogeneity is strikingly brought out. The regression lines for the yields of "paired replicated plots" are almost horizontal and show little or no correlation, whereas the regression lines for the yields of "paired contiguous plots" meet a horizontal at a marked angle and therefore show considerable correlation.

THE RELATION BETWEEN YIELDS OF OAT HAY AND WHEAT GRAIN

Harris⁸ has pointed out that differences between the yielding capacity of plots in a particular experimental field may persist for a period of years. In other words, there is a correlation between the yields of plots in successive years. Table 9 was prepared in order to determine whether there was such a relationship between the yields of oat hay based on the net areas of the plots and wheat grain based on the rod-row samples. The correlation coefficient obtained ($+0.364 \pm 0.036$) indicates that there is a significant correlation between the yield of oat hay in 1923 and the yield of wheat grain in 1924. Apparently there was less correlation between the yields of oat hay and wheat grain on the same plots in the two successive years the experiment was carried on than there was between yields of contiguous plots in either of the two years. This undoubtedly was due to seasonal influence and the fact that one-year yields were expressed in terms of oat hay and the other year in terms of wheat grain.

TABLE 9.—*Correlation of yield of wheat grain in 1924 with yield of oat hay in 1923 at Maggie, W. Va.*

	Oat hay, pounds per acre																
	1,100	1,300	1,500	1,700	1,900	2,100	2,300	2,500	2,700	2,900	3,100	3,300	3,500	3,700	3,900	4,100	
Wheat grain, bushels per acre:																	
7.5				1			1										2
9				4	2	1											7
10.5	1	2	1	7	6	4	3										24
12		2	5	8	10	6	3										34
13.5		1	5	14	4	2	2	3									31
15	1		6	17	15	7	3	1	1								51
16.5			5	18	11	7	1		1								43
18			3	11	14	5	1										34
19.5			2	5	6												15
21			2	6	2	3	1	1		1			1				17
22.5									1								3
24					1		2		1								2
25.5						1		1									2
27							1		1								1
28.5								1									3
30						1										2	
	2	5	29	91	71	39	17	5	6	2	0	0	1	0	0	0	270

$$r = 0.364 \pm 0.036.$$

Certain other correlation coefficients showing the relationship between yields of oat hay and wheat were also calculated. The coefficients obtained for the yield of oat hay and each of the following wheat yields—calculated grain on the entire plot, grain plus straw based on rod-row samples, and grain plus straw determined from the entire plot—were $+0.035 \pm 0.035$, $+0.385 \pm 0.035$, and $+0.394 \pm 0.035$, respectively. The magnitudes of these coefficients of correlation, and of the one mentioned in the preceding paragraph as well, are practically of the same order and do not differ significantly from one another.

⁸ HARRIS, J. A., and SCOTFIELD, C. S. PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD. Jour. Agr. Research 20: 335-356. 1920.

SUMMARY AND CONCLUSIONS

An experimental field at Maggie, W. Va., containing 270 plots, each with a gross area (68×21 feet) of approximately $\frac{2}{3}$ of an acre, was studied to determine the degree of soil heterogeneity that existed. As a basis for this study the yield of oat hay in 1923 and wheat grain in 1924 was used.

The yield of air-dry oat hay was determined by weighing the production of the entire net area (61×14 feet, or $\frac{1}{3}$ of an acre) of each plot. The yield of wheat in bushels per acre, and the weight of the straw plus grain, were determined from five rod rows removed from certain places in each plot. The air-dry weight of the total grain plus straw produced on the net areas of each plot was also determined.

The five rod rows of wheat showed a high correlation ($+0.951 \pm 0.004$) between the weight of the grain and the weight of straw plus grain. A high correlation ($+0.841 \pm 0.012$) was also found between the weight of the grain plus straw on the entire net area of each plot and the weight of the grain produced by rod-row sample removed from each plot. It was considered that yield based on a sample of five rod rows removed from a plot in the manner described constituted a fairly trustworthy index as to the yield of that plot.

A marked correlation was found between the yields of oat hay in contiguous plots, both when calculated in the usual way and when calculated by the formula suggested by Harris. The coefficients of correlation ranged from $+0.542 \pm 0.030$ to $+0.694 \pm 0.031$. The coefficients of correlation between yields of replicated plots were $+0.134 \pm 0.041$ and -0.068 ± 0.059 .

As in the case of oat hay, the wheat grain showed a marked correlation between the yields of contiguous plots ($r = +0.516 \pm 0.031$ to $r = +0.617 \pm 0.037$) and slight correlation between the yields of replicated plots ($r = +0.246 \pm 0.039$ and $r = +0.037 \pm 0.059$).

In the case of oat hay and of wheat grain there was somewhat less difference between the coefficients of correlation for contiguous plots and replicated plots when calculated by Harris's formula than when calculated in the ordinary way. The correlation coefficients for the yields of replicated plots by the ordinary method of calculation were sensibly zero.

It is concluded that the particular experimental field under observation is not naturally uniform with respect to productivity. Plot replication is effective in equalizing yield differences due to soil heterogeneity, and thus helps to overcome vitiating "place effect" in field experiments such as the one contemplated here.

There was some tendency for the plots which produced relatively high yields of oat hay in 1923 to produce relatively high yields of wheat grain in 1924. The correlation coefficient between the plot yields of these two successive years was $+0.364 \pm 0.036$.

A FORMULA FOR ESTIMATING SURFACE AREA OF DAIRY CATTLE¹

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INTRODUCTION

It has long been accepted as a fact that heat production in the animal body is due to the oxidation processes within the body, and hence is an accurate measure of the rate of metabolism. In order to utilize this standard in research, it becomes necessary to devise a satisfactory method for comparing the heat production of animals of different sizes.

Very early in nutritional investigations it became apparent that that the heat production of an animal is not proportional to its live weight. In 1848 Bergmann (3)^{3 4} attempted to explain the relatively higher heat production of smaller animals per unit of weight by the generalization that the heat production of the animal body is proportional to its surface area. This idea found wide application and was given strong support by many investigators.

Regnault and Reiset (10, p. 514)⁵ in their studies of the respiratory exchange of different species under diverse conditions, determined that the oxygen consumption of animals is not proportional to their weight. Obviously the heat production would have a similar ratio per unit of weight, which they explained as being due to the fact that small animals expose a relatively greater surface area to the cooling effect of the atmosphere and consequently require a greater heat production to maintain their body temperature. Some years later Rubner⁶ proved that their explanation was faulty, although their general statement of facts was correct.

Thus far the idea of the relation between heat production and surface area had been entirely theoretical, since no actual measurements of the surface area of living subjects were available. In 1879 Meeh (8) published the actual measurements of the surface area of living subjects, the results of his own painstaking work, which included the measurements of 6 adults and 10 children.

From his measurements Meeh developed a formula by which the surface area of any individual could be determined. This formula

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² The writer wishes to express his appreciation to A. C. Ragsdale, Professor of Dairy Husbandry, and to Samuel Brody, Assistant Professor of Dairy Chemistry, University of Missouri, for aid in outlining the problem and for helpful suggestions in interpreting and summarizing data and in the preparation of the manuscript. The major credit for developing the idea and details of the revolving metal cylinder of the measuring instrument, is due Samuel Brody. In this he was assisted by the writer. A. C. Ragsdale, C. W. W. Turner, and Chester Sparrow (an engineering student) all had a part in developing the plan and in perfecting this new method of measuring the surface area of animals. All experimental data were taken by the author and have been presented by him in a thesis submitted at the University of Missouri in partial fulfillment of the requirements for the degree of master of arts.

³ Reference is made by number (italic) to "Literature cited", p. 278.

⁴ Cited by Benedict (1).

⁵ Cited by Lusk (7, p. 120).

⁶ Cited by Lusk (7, p. 121).

was based on the fundamental mathematical law that the surface areas of similar solids are proportional to the two-thirds power of their volumes.

Assuming that the specific gravity was the same in each case, he substituted weight for volume so that his formula is expressed in the following form:

$$S = KW^{2/3}$$

in which S is the surface area in square centimeters, W the weight in kilograms, and K a constant (12.312 for adults and 11.9 for children).

In 1883 Rubner (11) measured the surface area of a number of dogs, varying greatly in weight, and also determined their heat production under comparable conditions. He found the body heat production to be quite constant per square meter of the body surface, but varying greatly per kilogram of body weight. A few years later, observations by Voit (13) indicated that this law is applicable over a wide range in the animal kingdom. He determined the heat production of subjects varying in size from a mature horse to a mouse and found the heat production per meter of body surface to be quite constant in all cases.

In 1916 D. Du Bois and E. F. Du Bois (4) measured the surface area of a number of human subjects, and from their measurements they developed a formula for estimating the surface area of humans based on the weight and height of the individual.

In general, investigators both in human physiology and pathology and in animal production have accepted the practice of calculating heat production per unit of surface area, and consequently surface area is the most common unit of reference in estimating basal metabolism.

Benedict (5, *p. 129*) and his coworkers have challenged this practice of calculating heat production per unit of body surface. They maintain that the heat production depends upon the actual mass of protoplasmic tissue within the body and not upon the cooling on the body surface. They have published an extensive series of prediction tables for determining heat production based on weight, height, sex, and age, but involving no assumption concerning derivation of surface area.

Extensive comparisons of the Benedict standard and the body-surface standard of Du Bois, show that the results obtained are almost parallel. More recently Benedict (2, *p. 159*) stated: "We believe that the accurate measurements of body-surface made possible by Du Bois may legitimately be used in a manner heretofore never practicable in metabolism experiments, provided that they are considered as physical measurements and with no erroneous conceptions as to the existence of a causal relationship between surface area and heat production."

Assuming that the surface area of the body is an accurate standard for estimating the metabolism, the investigator meets with difficulty because it has been impossible to calculate the surface area of animals with any degree of accuracy.

Earlier investigators have accepted the Meeh formula as applying to all types of animals, but the correct constants have been worked out in only a few instances, and also later investigations proved that in many instances this formula gave very erroneous results.

Trowbridge, Moulton, and Haigh (12) published a number of measurements of the surface area of cattle and calculated the constant for the Meeh formula. The constants varied from 7.319 to 10.74, depending on the age and the degree of fatness of the animals.

In 1916 Moulton (9) developed two formulas for estimating surface area of cattle, based on warm empty weight, but using a different exponent than the one used by Meeh. His formula for fat cattle is:

$$A = 0.158W^{\frac{2}{3}}$$

and for other animals:

$$A = 0.1186W^{\frac{2}{3}}$$

in which A is the surface area in square meters, and W is the empty weight in kilograms.

Hogan and Skouby (6) considered the work of Trowbridge, Moulton, and Haigh, and that of Moulton, as proof that the surface area of cattle could not be accurately calculated as a power function of weight, and they developed a formula for estimating surface area of cattle and swine, patterned after the height-weight formula of Du Bois (4), in which both weight and body length are factors. Their formula is:

$$S = W^{.4} \times L^{.6} \times K$$

in which S is surface area in square centimeters, W the live weight in kilograms, L the length of the body in centimeters, and K a constant (217 for cattle and 175 for swine).

The purpose of the work set forth in this paper was to actually measure the surface area of normal dairy cows, and to develop a simple formula for estimating the surface area of dairy cattle.

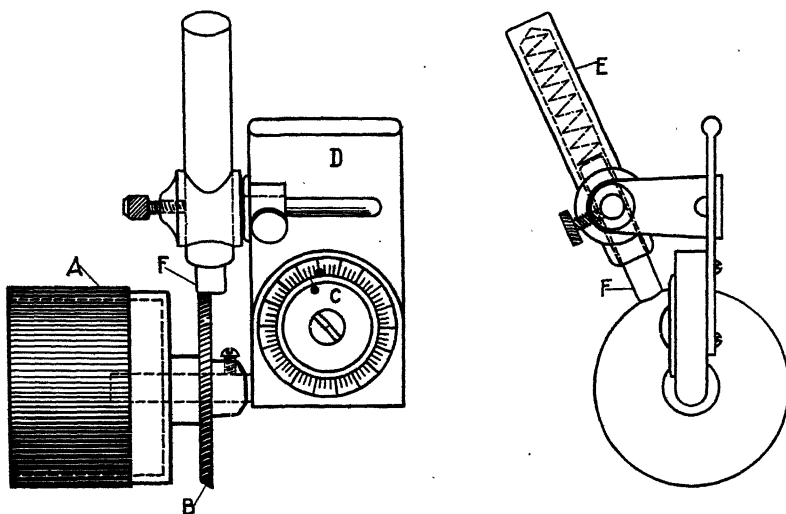
MEASUREMENT OF SURFACE AREA

It was rather difficult to find a satisfactory method for measuring the surface area of living animals with a fair degree of accuracy. The method finally used consisted in rolling a revolving metal cylinder of known area, attached to a revolution counter, over the entire surface of the animal, and then simply multiplying the number of revolutions of the roller by the area of the roller. This gave an approximately accurate surface-area measurement with a very small amount of computation, and the measuring instrument was not difficult to use.

The technique involved in taking surface area was simple. In most cases, only half of the body surface the right side of the animal was actually measured, and the results were multiplied by two. First, the dorsal and ventral median lines were marked with colored crayon. Then, starting at the base of the horn, the roller was passed along the dorsal median line to the posterior mid line. Lower down on the side of the animal, measurement was from the outline of the jawbone to the posterior mid line. The roller was equipped with a crayon marker which plainly marked the path of the roller. Then, by keeping the outer edge of the roller along the mark made the previous trip, the entire surface was covered. The only region which was difficult to measure accurately was around the udder and inside of the hind leg, but with a little extra care this was accomplished very satisfactorily. The legs were measured by moving the roller spirally

down them. The head and ears were measured last. The area of the tail was not measured with the roller, but it was estimated by multiplying the length, a figure representing the average of the diameters at the root and at the base of the switch.

Figures 1 and 2 are illustrations of the measuring apparatus. *A* is the brass cylinder. This roller is 2 inches long and 2 inches in diameter. Repeated trials with rollers of different lengths and diameters established this one as most satisfactory. A smaller roller did not turn uniformly, and a larger one was inconvenient in measuring around the flank, udder, armpit, etc. *B* is the milled rim of a disk which served as a marker. *C* is the dial of the revolution counter. *D* is the handle. *E* is a metal tube containing a fine spring which holds the crayon against the milled rim of the marking



SURFACE INTEGRATOR

FIG. 1.—Instrument used for measuring surface area of animal. *A*, brass cylinder; *B*, milled rim of disk which makes a chalk line; *C*, revolution counter; *D*, handle; *E*, metal tube containing spring; *F*, chalk crayon. (Drawing from photograph)

disk. *F* is the crayon. The milled rim made a fine but distinct chalk line on the animal. Figure 3 is a photograph of the measuring operation.

An effort was made to determine the quantitative error in the measured area as compared to the actual surface.

Two animals—cow 129 and heifer 27—were slaughtered. Both animals were measured with the roller on the morning before they were slaughtered. After they were slaughtered, the outlines of the hides were traced on paper and the areas of the tracings were measured. The results obtained are as follows:

Cow 129

Body area of live cow	42,635 sq. cm.
Area of hide	42,883 sq. cm.
Difference	+248 sq. cm.
Per cent difference (based on area of hide)	+0.58 per cent

	<i>Heifer 27</i>	
Body area of live heifer.....	14,821 sq. cm.	
Area of hide.....	14,701 sq. cm.	
Difference.....	—120 sq. cm.	
Per cent difference (based on area of hide).....	—0.82 per cent	

The two methods gave approximately the same results.

DESCRIPTION OF ANIMALS USED

The data presented in this paper were all taken on Holstein and Jersey animals. Practically every animal of these two breeds in the University of Missouri dairy herd have been measured, and in some cases, in order to obtain more data, certain young animals were measured two or more times at different ages. The data include

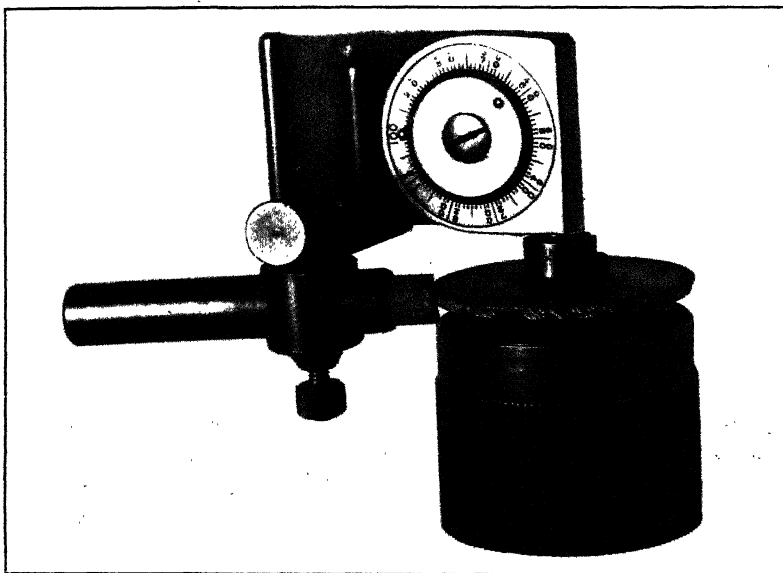


FIG. 2.—Photograph of instrument for measuring surface area of animal

measurements of animals varying in age from a few hours to 10 years, and in weight from 17.6 to 653 kilograms. The animals represent many variations in degree of fleshing, body conformation, and stages of lactation and gestation.

LIVE WEIGHT OF ANIMALS

In all cases the weight of the animal was taken immediately before the surface area was measured. No attempt was made to withhold either feed or water before weighing.

DEVELOPMENT OF FORMULA

When the live weight was plotted against surface area, as in Figure 4, a simple parabolic curve was obtained with all the points falling reasonably close to the curve. Such a curve indicated that surface area is a direct power function of weight, and hence may be determined by a simple formula as

$$X = Ky^n$$

By plotting surface area against weight on logarithmic paper as in Figure 4, a straight line was obtained having a slope of 0.56 which designated the value of the exponent n in the equation. In general, the values for the Holsteins were slightly greater than those for Jerseys (fig. 4). In reality, the value 0.57 for Holsteins and of 0.55 for Jerseys is more nearly exact than the average figure 0.56, but a few trial calculations indicated that the slight increase in accuracy of results obtained by using a different exponent in the formula for the different breeds was not sufficient to justify the use of two separate formulae. Knowing the live weight and the surface area of the animals and the value of n , it remained to solve for K in the formula. By choosing values that fell on the weight-surface area curve, and substituting them in the formula, it was possible to solve for K .

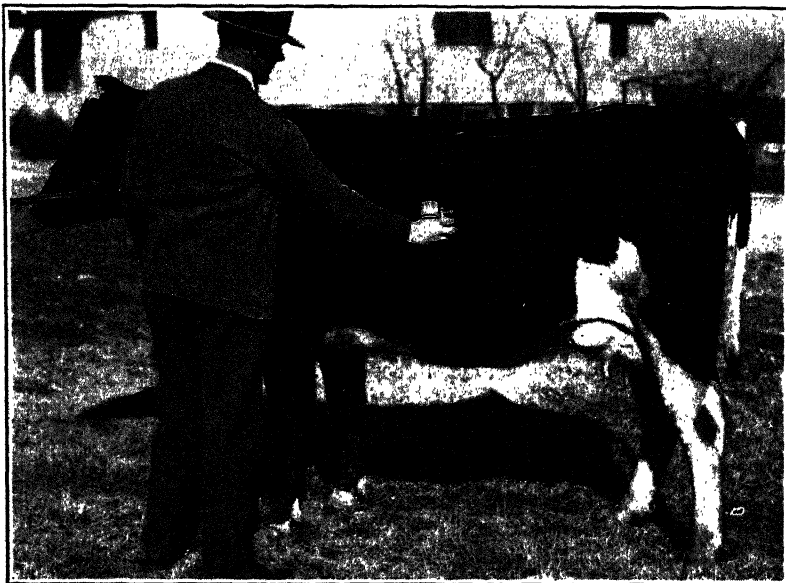


FIG. 3.—Measuring the surface area of a cow, with the instrument shown in Figures 1 and 2

$$S A = K W^n \text{ or } \frac{S A}{W^n} = K$$

Using logarithms, the equation becomes

$$\text{Log } S A - 0.56 \text{ Log } W = \text{Log } K$$

The value of K was 1,470. Then the formula in its final form becomes

$$S A = 1,470 W^{0.56}$$

in which $S A$ is the surface area in square centimeters, W is the live weight in kilograms, and 1,470 is a constant.

The values of W for the different animals were substituted in the formula, and the equations solved to determine the surface area. These computed values for surface area were then compared with the observed values. These results are given in Table I. In all but 4

cases, or in 96 per cent of all animals measured, the computed value was within ± 5 per cent of the observed value. The remaining 4 animals had computed values varying from 5.4 per cent to 6.17 per cent above the observed values. The average per cent deviation in all animals, regardless of sign, was 1.95. Among the 46 animals having a

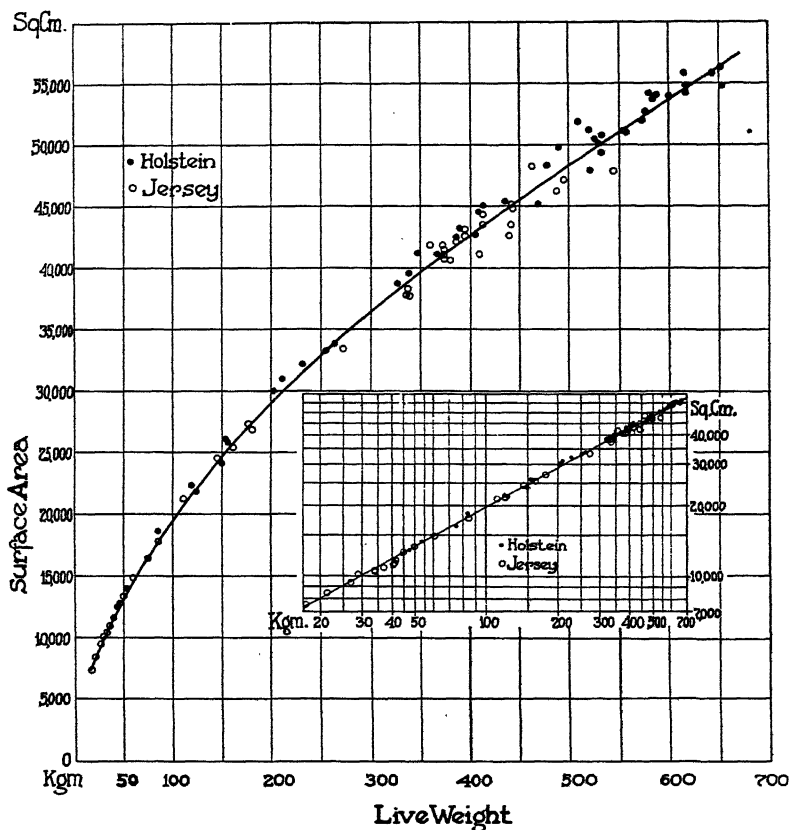


FIG. 4.—Relation between the live weight and the surface area of dairy cows

computed value greater than the observed value the average per cent of deviation was 1.99, and among the 50 animals having a computed value lower than the observed value the average per cent of deviation was 1.92.

The area was also computed according to the Meeh formula (8). These results are given in Table I in order that they may be compared with the values obtained from the new formula.

TABLE 1.—Data used in developing formula and computed values

JERSEYS							
Herd No. of animal ^a	Age of animal	Live weight, kgm.	Surface area				
			Measured Surface, sq. cm.	Computed			
				S=1470 W ^{0.56}		S=839 W ^{2/3}	
				Surface, sq. cm.	Error, per cent	Surface, sq. cm.	Error, per cent
1.	10 hours	17.6	7,296	7,375	1.08	5,676	-22.20
2.	2 days	21.5	8,433	8,232	-2.38	6,466	-23.33
170.	3 days	27.0	9,404	9,421	0.18	7,587	-19.32
187.	16 days	29.0	10,076	9,767	-3.06	7,818	-21.41
189 ¹ .	23 days	34.0	10,557	10,581	1.17	8,803	-16.61
188 ¹ .	35 days	34.0	10,395	10,681	2.76	8,803	-15.31
189 ² .	38 days	40.5	11,388	11,785	3.66	9,893	-12.97
188 ² .	40 days	41.5	11,692	11,980	2.46	10,088	-13.71
186 ¹ .	44 days	41.0	11,469	11,867	3.47	9,974	-13.03
157 ¹ .	50 days	34.0	10,443	10,681	2.27	8,803	-15.70
157 ² .	70 days	37.0	10,954	11,201	2.25	9,314	-14.97
164.	80 days	50.0	13,466	13,267	-1.47	11,381	-15.48
185 ¹ .	72 days	45.0	12,671	12,505	-1.31	10,613	-16.24
27.	3 months	61.0	14,821	14,839	0.12	12,997	-12.30
182 ¹ .	4 months	85.0	17,892	17,883	-0.05	16,223	-9.32
181 ¹ .	5 months	111.0	21,291	20,779	-2.40	19,373	-9.00
186 ² .	6 months	122.0	21,672	21,915	1.12	20,633	-4.79
183.	6 months	161.0	25,510	25,614	0.41	24,823	-2.69
185 ² .	6 months	145.0	24,540	24,160	-1.54	23,150	-5.66
182 ² .	7 months	177.0	27,294	27,016	-1.02	26,359	-3.42
182 ² .	8 months	179	26,817	27,188	1.38	26,640	-0.66
181 ² .	9 months	181	26,970	27,358	1.44	26,753	-0.80
180.	12 months	272	33,423	34,401	2.92	35,209	5.34
150.	1 year, 4 months	336	37,853	38,744	2.35	40,395	6.71
177.	1 year, 7 months	324	38,964	37,959	-2.57	38,664	-0.77
176.	1 year, 7 months	336	38,385	38,744	0.98	40,537	5.66
173.	1 year, 10 months	374	41,051	41,151	0.24	43,536	6.05
172.	1 year, 11 months	395	43,094	42,435	-1.53	45,151	4.77
171.	1 year, 11 months	359	41,959	40,215	-4.15	42,364	0.96
170.	2 years, 1 month	413	43,544	43,512	-0.07	45,512	4.51
166.	2 years, 7 months	374	40,750	41,151	0.98	43,536	6.83
167.	2 years, 7 months	372	41,822	41,027	-1.90	43,382	3.73
165.	2 years, 8 months	381	40,609	41,583	2.39	44,078	5.54
164.	2 years, 11 months	338	37,773	38,874	2.91	40,696	7.73
157.	4 years, 1 month	374	41,424	41,151	-0.65	43,536	5.10
156.	4 years, 1 month	395	42,595	42,435	-0.37	45,151	6.00
154.	4 years, 10 months	463	48,283	46,401	-3.85	50,575	4.79
151.	5 years, 3 months	545	47,900	50,858	6.17	55,958	16.82
129.	7 years	442	42,635	45,205	6.02	48,694	14.18
126.	7 years, 4 months	495	47,161	48,179	2.15	52,482	11.28
127.	7 years, 7 months	444	44,850	45,320	1.02	48,811	8.83
120.	7 years, 10 months	386	42,135	41,889	-0.58	44,463	5.52
121.	8 years	442	43,430	45,205	4.08	48,665	12.05
110.	8 years, 1 month	487	46,189	47,739	3.35	51,913	12.39
125.	8 years, 9 months	442	45,106	45,205	0.21	48,666	7.89
108.	9 years	410	41,112	43,334	5.40	46,287	12.58

^a Superior numbers indicate animals measured more than one time.

TABLE 1.—Data used in developing formula and computed values—Continued

HOLSTEINS								
Herd No. of animal	Age of animal	Live weight, kgm.	Surface area					
			Measured Surface, sq. cm.	Computed				
				S=1470 W ^{0.56}		S=839 W ^{2/3}		
				Surface, sq. cm.	Error, per cent	Surface, sq. cm.	Error, per cent	
552	1 day	47.5	12,847	12,907	0.46	11,017	-14.24	
274	3 days	53.5	13,982	13,782	-1.43	11,910	-14.81	
554	7 days	41.0	11,600	11,867	2.30	9,974	-14.01	
553	9 days	44.5	12,573	12,411	-1.28	10,517	-16.35	
550	7 weeks	75.0	16,466	16,667	1.22	14,922	-9.37	
551	3 months	84.0	18,660	17,764	-4.80	16,087	-13.78	
549	3½ months	120.0	22,376	21,611	-3.42	20,406	-8.80	
548	4 months	125.0	21,817	22,210	1.80	20,847	-4.45	
545	4½ months	150.0	24,061	24,615	2.30	23,668	-1.63	
546	5 months	154.0	26,159	24,981	-4.50	24,105	-7.85	
547	6 months	156.0	25,820	25,209	-2.36	24,306	-5.86	
544 ¹	7 months	202.0	30,081	29,235	-2.81	28,875	-4.00	
544 ²	7½ months	208.0	31,041	29,663	-4.43	29,444	-5.14	
542	8 months	231.0	32,260	31,381	-2.72	32,709	1.39	
541	10 months	254.0	33,335	33,102	-0.69	33,528	0.58	
540	1 year	263.0	33,870	33,757	-0.33	34,315	1.31	
537 ¹	1 year, 2 months	336.0	39,591	38,744	-2.14	40,646	2.66	
534	1 year, 4 months	327.0	38,680	38,157	-1.35	39,824	2.95	
535	1 year, 4 months	408.0	44,499	43,216	-2.88	46,136	3.67	
537 ²	1 year, 5 months	389.0	43,194	42,062	-2.62	44,533	3.09	
536	1 year, 6 months	469.0	45,242	46,728	3.28	50,626	11.90	
533	1 year, 6 months	413.0	45,091	43,512	-3.50	46,515	3.15	
538	1 year, 6 months	406	42,604	43,096	1.15	45,986	7.13	
532	1 year, 7 months	368	41,076	40,778	-0.72	43,086	4.89	
531	1 year, 8 months	347	41,200	39,446	-4.25	41,432	0.56	
530	1 year, 8 months	386	42,495	41,889	-1.42	44,481	4.67	
527	1 year, 10 months	436	46,384	44,859	-1.15	48,253	6.32	
526	2 years, 2 months	533	50,841	50,224	-1.21	55,133	8.44	
525	2 years, 5 months	553	51,165	51,276	0.22	56,503	10.43	
521	2 years, 11 months	644	55,925	55,864	-0.11	62,570	11.88	
518	3 years, 1 month	531	50,138	50,119	-0.04	55,017	9.73	
517	3 years, 9 months	601	54,044	53,733	-0.58	59,752	10.56	
515	4 years	616	55,937	54,185	-3.13	60,345	7.88	
512	4 years, 2 months	508	51,914	48,885	-5.83	53,395	2.85	
510	4 years, 3 months	576	52,737	52,465	-0.52	58,083	10.14	
509	4 years, 3 months	490	49,805	47,904	-3.81	52,149	4.70	
508	4 years, 5 months	478	48,319	47,241	-2.23	51,293	6.15	
507	4 years, 6 months	574	52,012	52,362	0.67	57,950	11.41	
503	5 years	617	54,334	54,535	0.37	60,809	11.91	
288	6 years	579	54,240	52,620	-2.98	58,261	7.41	
290	6 years	653	54,818	56,302	2.71	63,151	15.20	
292	6 years	592	54,172	53,281	-1.64	59,154	9.19	
285	6 years, 2 months	522	47,883	49,640	3.66	53,158	11.01	
281	6 years, 7 months	526	50,521	49,852	-1.32	54,672	8.21	
281	6 years, 8 months	556	51,008	51,432	0.83	56,731	11.21	
279	7 years, 4 months	576	52,805	52,466	-0.64	58,083	10.00	
274	7 years, 5 months	608	53,710	54,085	0.70	60,214	12.11	
275	7 years, 5 months	520	51,278	49,532	-3.40	54,254	5.80	
266	8 years, 5 months	535	49,399	50,331	1.88	55,293	11.93	
254	10 years	617	54,830	54,534	-0.53	60,810	10.90	

DISCUSSION

A comparison of the computed values by the two formulae shows that a much greater range of error was obtained by using the two-thirds power of weight than by using the 0.56 power. The former gave results which were much too low in very small animals, and much too high in large animals, the percentage of error ranging from -23.2 per cent in 2-day-old calf to +16.8 per cent in a very fat barren cow. It was found that the constant 839 gave the most uniform results, but even then the range of error was so great that such results are of little value.

It is hardly fair to compare results obtained by the new formula with those obtained by Moulton's formula (9), inasmuch as his formula is based on warm empty weight rather than live weight. A few trial computations, substituting live weight for W in Moulton's formula, showed that when the five-eighths power of weight is used the results again are too low in very small animals and too great in large animals, especially in those which are rather fat.

The results obtained by using the 0.56 power of weight were in close agreement with the observed values. Of the four animals whose computed areas varied more than 5 per cent from the observed areas three were abnormal individuals. Cows 129 and 151 were nonbreeders. Both were dry and very fat when measured. Cow 108 was a very compactly built, short-legged individual, which was dry and quite fat when measured.

The possibility of introducing a second variable factor into the formula was discarded, since it was found possible to determine surface area quite accurately as a simple power function of weight. A second variable would only complicate the formula without increasing its accuracy, because the probable error in the observed value makes it questionable whether results closer than ± 5 per cent are possible.

SUMMARY AND CONCLUSIONS

In normal dairy cattle, surface area has been found to be a direct power function of weight. The formula $SA = 1470 W^{0.56}$, in which SA is the surface area in square centimeters, and W the live weight of the animal in kilograms, accurately expressed the relationship between surface area and live weight.

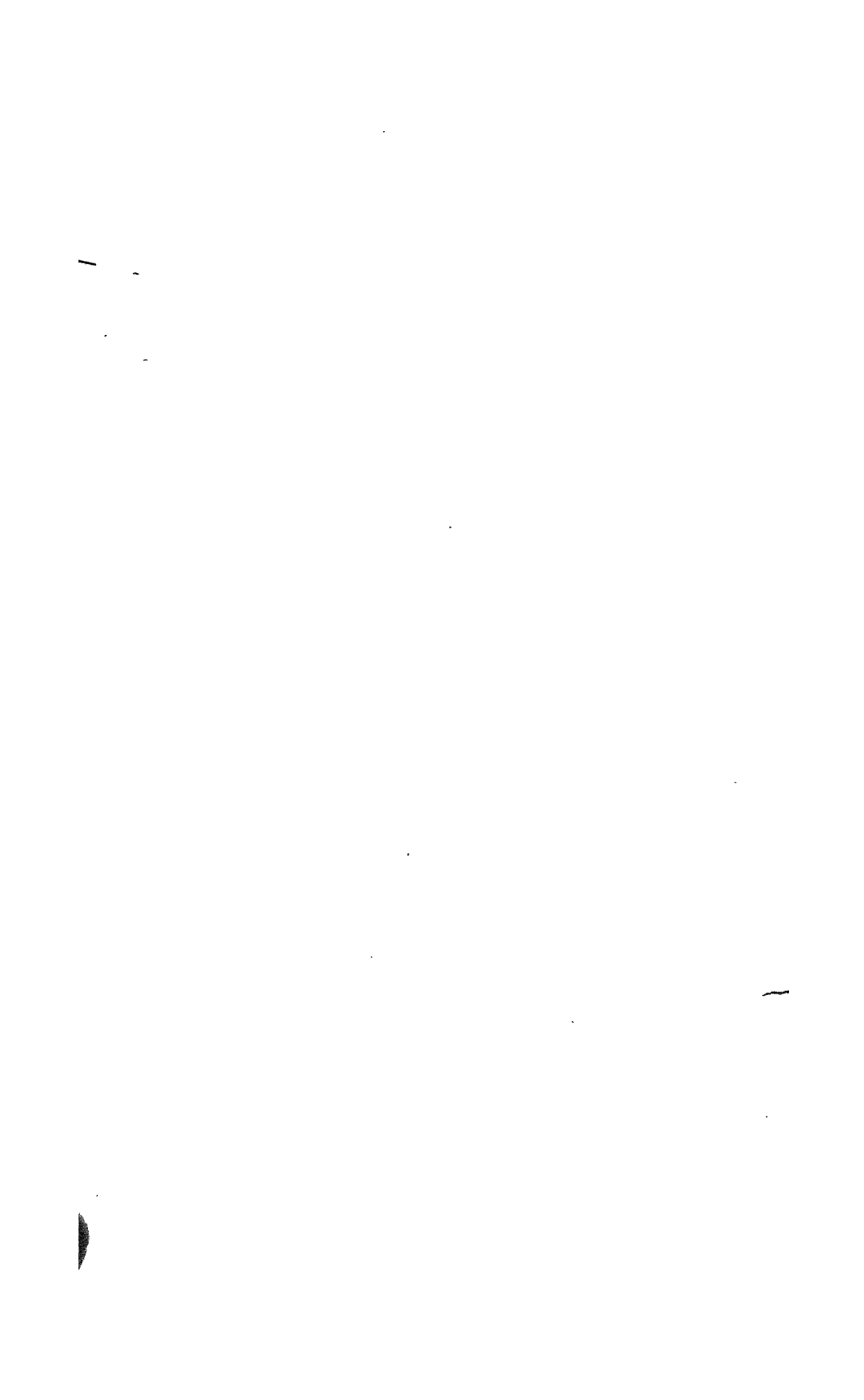
Both the two-thirds and the five-eighths powers of the weight gave results which were much too low in very small individuals, and much too high in large or extremely fat animals.

The surface areas of 96 dairy cattle were measured. The areas of 92 animals as computed by the proposed formula were within ± 5 per cent of the observed areas, and the maximum error in the case of an extremely fat barren cow was only 6.2 per cent. The introduction of linear measurements into the formula would only complicate it without increasing its accuracy.

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THE CAUSE AND CONTROL OF YELLOW BERRY IN TURKEY WHEAT GROWN UNDER DRY-FARMING CONDITIONS.¹

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INTRODUCTION

Under a cooperative agreement between the Bureau of Plant Industry of the United States Department of Agriculture and the Oregon Agricultural Experiment Station, a farm of 230 acres at Moro, Oreg., has been operated as a dry-land experiment station since 1911. Located in a region in which the rainfall is between 12 and 13 inches, its sole purpose is to solve problems that are peculiar to the dry-farmed areas of the Central Columbia River Basin.

The typical dry-farmed lands in this region are rich in the mineral elements of plant food. In composition, their weakness lies in their limited amount of native organic matter, which in time must fail to yield, in ordinary processes of decomposition, the requisite amount of available nitrogen to meet fully the crop requirements in the matter of growth. Since climate and rainfall practically limit the cropping of the dry-farmed lands to the small grains, no satisfactory means are apparent as yet for guarding against ultimate depletion of native organic matter and nitrogen.

TILLAGE EXPERIMENTS

Early in the development of experiment station work at Moro, a series of tillage plots was laid out for the purpose of determining what might be the effect on the accumulation of soil moisture and grain yields of variation in the tillage practice of the summer fallow. The plan of the work will be understood readily from the arrangement of the plots shown in Figure 1, if while studying that arrangement it is noted that each year while three tiers of plots of early spring, medium-early spring, and late-spring plowing were being summer-fallowed, three other adjacent tiers, identical plot for plot in previous summer-fallowed treatment, were in crop. All kinds of summer-fallow tillage were to be practiced, including extremes of thoroughly good and thoroughly poor. Turkey wheat was the crop to be grown.

EARLIER FIELD OBSERVATIONS

Although this series of plots was planned originally for the study of the effect on yields of various methods of summer-fallow treatment, it was noted as long ago as 1914 (7, 8) that grain from the differently treated plots varied strikingly in its percentage of yellow berry. Plots given good summer-fallow treatment produced not only the largest amount of grain and straw, but always grain with the least percentage of yellow berry.

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Normally, the kernels of Turkey wheat are hard and flinty. Cross sections of them are translucent in appearance, there being no visible indication of starch deposits. Yellow-berry kernels are lacking in hardness, and the clear, dark red color that is characteristic of Turkey wheat at its best. The mottled appearance is evidence of deteriorated quality. Upon cross-sectioning, starchy deposits are clearly evident. If mottling is very pronounced offerings may be docked several cents per bushel under prevailing prices for the best Turkey. Turkey wheat is the variety grown most in this particular region. It stands high in the estimation of producers and millers. Since yellow berry to the extent of 50 per cent or more is of frequent occurrence, it appeared from these earlier field observations on the

381	382	383	384	385	386	387	388	389	390
LATE SPRING-PLOWED (JUNE 1)									

281	282	283	284	285	286	287	288	289	290
MEDIUM-EARLY SPRING-PLOWED (MAY 1)									

181	182	183	184	185	186	187	188	189	190
EARLY SPRING-PLOWED (APRIL 1)									

FIG. 1.—Arrangement of tillage plots. This series alternates in fallow and crop with a similarly arranged series, numbered 481-490, 581-590, and 681-690

Nos. 181, 281, and 381, packed with surface packer, and frequently cultivated. Clean summer fallow.
 Nos. 182, 282, and 382, no cultivations of summer fallow after plowing. Weeds hoed off before maturity. Neglected fallow.
 Nos. 183, 188, 283, 288, 383, and 388, check plots, spring-disked before plowing. Clean fallow
 Nos. 184, 284, and 384, one harrowing after plowing. No further cultivation. Weeds hoed off before maturity. Neglected fallow.
 Nos. 190, 290, and 390, fall-disked and spring-disked before plowing. Frequent cultivation. Clean fallow. (Size of plots: 2 rods by 8 rods. Width of alleys between plots, 4 feet 7 inches. Width of roadway between tiers, 1 rod)

tillage plots that advantage might be taken of them—and without change in the original plans for them—to try to find the fundamental cause of yellow berry and means for lessening its prevalence.

GOOD AND POOR SUMMER FALLOW

"Good" summer-fallow treatment implies fall or early spring disking before plowing, reasonably early spring plowing and cultivation with harrow and weeder sufficient to keep the surface soil loose and the plot clean of weeds. It is just such treatment as the

thoroughgoing grower will give his summer fallow when determined to retain in it the greatest percentage of soil moisture possible. "Poor" summer-fallow treatment means no disking previous to plowing, late spring plowing, and little if any summer cultivation. It is the treatment given his summer fallow by one who, for one reason or another, fails to employ the best methods for conserving soil moisture.

Laboratory data on the crops of 1918 and 1919 confirmed an estimate one might have made readily from field observations as to the relative values for milling purposes of the grains from the various tillage plots. Flour millers place a premium on high content of protein in wheat, and in this respect the grain from the well-tilled plots had a decided advantage. These earlier field and laboratory observations are incorporated along with subsequent observations of like kind in Table 1.

TABLE 1.—*Field and laboratory data for crops grown on well-tilled and poorly-tilled summer fallow, 1918 to 1924, inclusive*

Year	Tillage treatment and average number of plots	Yields in pounds			Ratio of grain to straw	Physical characteristics of grain				Protein on dry basis	
		Total	Grain	Straw		Weight		Flinty, per cent by weight	Yellow berry, per cent by weight	In grain	In straw
						Per bushel	Per 1,000 kernels				
					<i>Lbs.</i>	<i>Gm.</i>			<i>P. ct.</i>	<i>P. ct.</i>	
1918	{Good, 7.....	4,714	1,241	3,473	1:2.8	57.6	20.3	98.6	1.4	17.32	
	{Poor, 7.....	3,744	1,281	2,463	1:1.9	60.0	24.6	61.7	38.3	11.69	
1919	{Good, 4.....	7,180	2,148	5,032	1:2.3	60.0	22.1	96.2	3.8	14.80	
	{Poor, 4.....	3,195	1,315	1,880	1:1.4	61.5	25.3	72.7	27.3	13.34	
1920	{Good, 6.....	5,930	1,615	4,315	1:2.7	58.3	23.3	99.3	.7	16.29	
	{Poor, 6.....	4,750	1,580	3,170	1:2.0	60.8	25.0	69.8	30.2	11.88	
1921	{Good, 6.....	8,395	2,103	6,292	1:3.0		26.3	44.8	55.2	10.72	
	{Poor, 6.....	5,302	1,563	3,739	1:2.3		30.8	15.0	85.0	8.99	
1922	{Good, 6.....	3,798	1,117	2,665	1:2.4	54.6	17.6	93.2	6.8	18.02	3.86
	{Poor, 6.....	2,490	913	1,577	1:1.7	57.8	22.0	61.5	38.5	12.54	2.32
1923	{Good, 8.....	8,646	2,016	6,630	1:3.3	59.7	23.9	79.9	20.1	11.65	1.93
	{Poor, 7.....	6,706	1,781	4,925	1:2.8	60.0	26.7	49.2	50.8	10.49	1.54
1924	{Good, 8.....	6,542	1,284	5,258	1:4.1	58.3	19.2	98.7	1.3	16.57	
	{Poor, 7.....	3,505	1,119	2,386	1:2.1	60.0	20.8	73.8	26.2	12.66	

LATER FIELD AND LABORATORY OBSERVATIONS

Field and laboratory observations subsequent to 1919 entirely confirm the earlier ones which induced this study of the cause and prevention of yellow berry.

There has developed a very marked difference in the habit of growth of plants on plots receiving good as compared with poor summer-fallow treatment. Although the rate of seeding is the same for all plots, the early-plowed and well-tilled ones invariably produce a thicker stand of heavily tillered plants of a deeper green color, greater height, and sturdier growth than the plots given late spring plowing and little or no cultivation during the summer-fallow season. Figure 2 was reproduced from a photograph of six plants uprooted April 25, 1921, from an early-plowed well-tilled plot, and of six taken at the same time from a late-plowed poorly tilled-plot. Occasionally soil moisture is insufficient to carry the heavier growth to maturity without damage to yields of straw and grain by burning.

As a rule, however, the good fallow has continued to produce the greater weight of straw and grain; the grain from those plots continues to be less infected with yellow berry, and both grain and straw are substantially richer in protein. These facts are summarized down to 1924 in Table 1.

CAUSE OF YELLOW BERRY

Anyone at all familiar with the history of hard-wheat production will recognize in the preceding statements of facts as observed for Turkey-wheat on the tillage plots at Moro, a problem that is as old as the hard-wheat industry. It may be well to refer to comments by European writers on the prevalence of yellow berry in the hard wheats of southeastern Europe, and to those of Australian investigators regarding its appearance in hard Australian wheats. In the United States and Canada, too, more or less notice has been taken of yellow berry in hard-wheat regions, and some speculation has been indulged in as to its cause and remedy. The importance attached to it is directly in proportion, of course, to the cut made by millers under the prevailing market price for normal grain of the same class. For the immediate purpose of this paper it is sufficient to cite only those more recent investigators who have expressed concrete suggestions as to cause and remedy, based upon experimental evidence.

Roberts and Freeman (5) in 1908 advanced the suggestion that yellow berry in the hard winter wheats of Kansas "is a 'tendency,' which finds expression in certain strains or races more markedly than in others, and is heritable." In a report of later work by Roberts in 1919 (6), it is apparent that he, while still convinced that his earlier suggestion was well-founded, felt obliged to concede that however pronounced heritable tendencies in the direction of yellow berry might be for any one variety of hard winter wheat, soil and climatic factors, operating for or against that infection, were far more pronounced in their effect.

In 1914 LeClerc and Yoder (4), of the Bureau of Chemistry, United States Department of Agriculture, mentioned "flinty kernels" in discussing environmental influences on wheat. From the results of then recently concluded experimental work they concluded that climate is the most potent factor in determining both the physical and the chemical properties of wheat. In reporting more recent work by the Bureau of Chemistry on the effects of nitrogen fertilization on wheat, Davidson and LeClerc (1) conclude that inorganic nitrogen applied at the time of heading is a very effective agent in preventing the development of yellow berry in hard wheat. Expression is also given by these investigators to the view that possibly the color of the flinty kernels is due to the residual effect of the basic element of the nitrogen compound.

Headden (2) comments upon the tendency of some observers to charge up the cause of yellow berry to excessive rainfall and, in irrigated districts, to excessive irrigation. He produced field and laboratory data to prove that variation of soil moisture within rather wide limits under irrigation practice is without effect on the development of yellow berry. He expressed the opinion, also, that far too much importance has been attached to climatic factors as

causal agents of yellow berry, and cites his own experiments in Colorado to prove that the all-important factor in the production of uniformly hard, flinty wheat, uninfected with yellow berry, is a proper ratio of available soil potassium and nitrogen. Headden's work was almost entirely with hard spring wheats.

The senior author of this paper and associates (3) at the University of Idaho have shown also that the hardest of hard spring wheats is both possible and reasonably to be expected with irrigation on improved sagebrush lands of the Snake River Plains region. ("Improved lands" in this connection means lands enriched with available nitrogen by the growth of alfalfa and red clover, for which crops the sagebrush lands are remarkably well adapted.)

One might readily conclude from the field and analytical data already given that there is already sufficient evidence that well-tilled plots produce better-nourished plants. The higher yields of grain and straw from these plots, and the higher content of nitrogen in both, simply mean that substantially larger amounts of nitrogen and, presumably, of other elements have been furnished the growing crop on well-tilled plots each year as far back, at least, as 1918. It has appeared worth while, however, to attempt at least to get down to the fundamental reasons for this result.

DRY-FARMED LANDS LOW IN ORGANIC MATTER

It has been remarked that the inherent weakness of the soils in this particular dry-farmed region lies in their low content of organic matter. Unless nitrogen fixation takes place much more extensively than is surmised, crops must depend on this small supply of organic matter for their supply of nitrogen. There is no evidence that this condition is at present the limiting factor in crop production. It is conceivable, however, that long before the soil's native supply of nitrogen is depleted sufficiently to make of that element the limiting factor in crop production, the lack of a liberal supply of that element in readily available form at all times might be reflected in some physiological disturbance in crop growth—as yellow berry in Turkey wheat.

The denser and more vigorous growth of plants on the early plowed and well-tilled plots might be due solely to a greater soil-moisture content conserved by better tillage, or it might be due to a larger content of soil nitrogen and other elements of plant food in readily available form. The first-mentioned condition might be, in part at least, the cause of the second, but only the larger supply of available plant food elements, particularly of nitrogen, could stimulate the greater production of protein in the grain and straw of the plots which make the denser growth. Soil-moisture determinations on these plots have been made systematically almost from the initial layout. In 1920 it was decided to try to find out whether differences already noted in yields, and more particularly in the character of the crop, could be correlated with significant differences in available soil nitrogen and possibly other elements of plant food. The summarized analytical data for five years seem to give a substantial basis for definite conclusions as to the cause of yellow berry in dry-farmed Turkey wheat.

MOISTURE AND NITRATE ACCUMULATIONS IN SUMMER FALLOW

Obviously the mass of analytical data resulting from work so wide in scope was necessarily great. However, each year the data were reduced to graphic form, and the trend of events was closely studied. As the year-by-year results warrant the same general conclusions, they are presented here in summarized graphic form only.

In all cases the composite samples taken from the plots for moisture and nitrate determinations represent four borings as the plots were



FIG. 2.—Growth of plants on two different plots. The plots were adjacent (see fig. 1), but received different summer-fallow treatment. These plants were uprooted April 25, 1921. *Top row:* Representative plants from plot 383, plowed April 1, 1920, and given frequent cultivation during the fallow season. *Bottom row:* Representative plants from plot 382, plowed June 1, 1920, and given no cultivation during the fallow season.

traversed from end to end. Graphs for each of the 6 feet might have been reproduced here, but those representing the averages presented are sufficient for the immediate purposes of this paper.

In Figures 3 and 4, beginning with fallowed and cropped land, respectively, is shown the course of events with respect to soil moisture in 6 feet of typically "good" and "poor" fallowed plots through a continuous period of five years.

One must note the greater decline in soil moisture in the poorly tilled plots during the period of no rainfall during the summer-fallow season; the sharp rise in soil moisture in all plots coincident with the late summer and fall rains; the near equality of soil moisture in all plots in the early spring of the cropped year; and the very sharp decline in soil moisture in all plots accompanying crop growth to near equality at harvest time.

The slightly greater moisture content of the poorly tilled plots during the summer-fallow season from seed formation to harvest time is

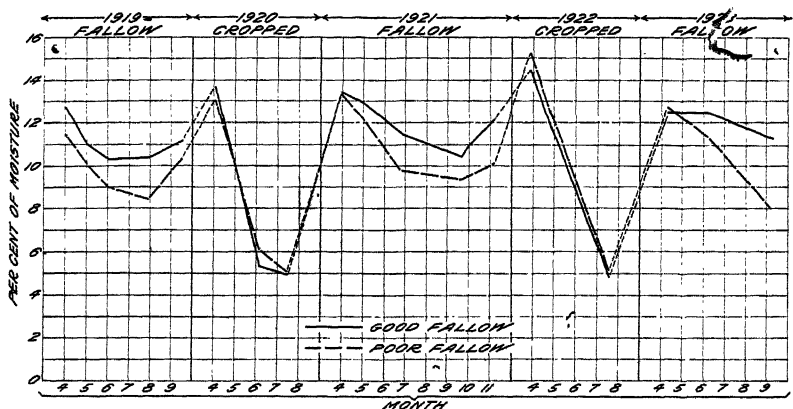


FIG. 3.—Soil moisture in 6 feet of typically "good" and typically "poor" fallowed plots through a continuous period of five years. Fallowed in 1919

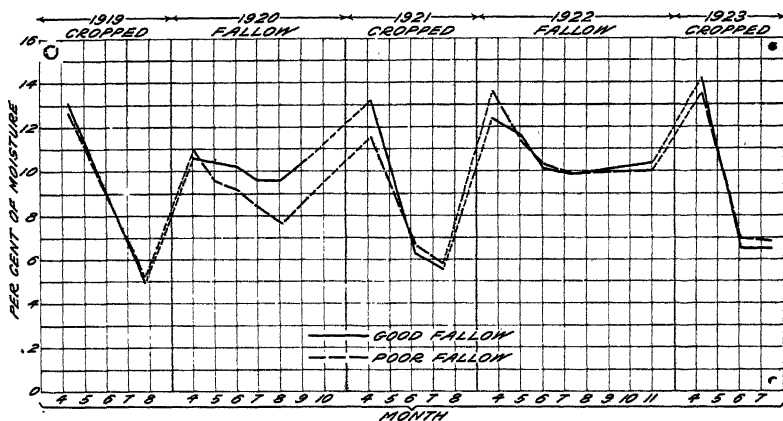


FIG. 4.—Soil moisture in 6 feet of typically "good" and typically "poor" fallowed plots, through a continuous period of five years. Cropped in 1919

characteristic. So also is the greater percentage of yellow berry in the harvested grain of these plots. It is believed, however, that the first difference, however characteristic, is entirely too small to account in any appreciable measure for the latter. There seems to be more evidence for supposing that the greater moisture content of the well-tilled plots during the summer-fallow season is conducive to greater bacterial activity, the sum total of which is a larger amount of available plant food in readiness for the succeeding crop.

NITRATES IN THE CROPPED PLOTS

The first attempts to establish the correctness of this view were made in 1920. Differences in habits of growth previously noted were so very marked in the early springtime that determinations of soil nitrates on the cropped plots seemed promising of results that would establish a connection between available soil nitrogen and vigor of crop growth. From Table 2 it will be seen that although the actual amounts of nitrogen in nitrate form were low on April 15 and May 5, the well-tilled plots had decidedly the larger accumulation of excess available nitrogen. By May 19 the differences were practically negligible as the natural result of the more exhaustive feeding of the denser growth on the well-tilled plots. It is evident that by May 19 there was no appreciable excess of available nitrogen in either well-tilled or poorly tilled plots. All subsequent nitrate determinations were confined to plots actually in summer fallow. This introduces some difficulties also in the matter of interpretation and correlation of analytical data.

TABLE 2.—Average nitrate content per foot of soil to a depth of 6 feet in plots under crop in 1920

[Some plots have from the start received good tillage while under summer fallow; others have been given little or no tillage during the fallow season]

Date of sampling	Good tillage		Poor tillage	
	Plot No.	NO ₃ , parts per million	Plot No.	NO ₃ , parts per million
Apr. 15.....	481	3.88	482	1.03
	581	5.16	582	1.66
	681	4.98	682	1.66
	483	3.00	484	2.19
	583	4.04	584	2.02
	683	3.16	684	2.49
Average.....		4.04		1.84
May 5.....	481	15.5	482	5.75
	581	7.5	582	3.80
	681	10.0	682	4.60
	483	7.25	484	7.02
	583	11.30	584	3.88
	683	8.40	684	4.57
Average.....		9.99		4.94
May 19.....	681	5.67	682	4.65
	683	4.25	684	4.10
Average.....		4.96		4.38

NITRATE ACCUMULATION IN FALLOWED PLOTS

Figures 5 and 6 were derived from moisture and nitrate data indicative of the average foot for a depth of 6 feet in well-tilled and poorly-tilled plots from 1920 to 1924, inclusive. Figures 7 and 8 were derived from data of the same kind for the first foot of soil. Inasmuch as one will arrive at essentially the same conclusions from a study of both sets of figures, comment will be made on only the latter. From the figures at least four facts can be read:

(1) There is a near equalization of nitrates in all plots in early spring; (2) the period of most rapid nitrate formation roughly coin-

cides with the period of soil moisture decrease and temperature rise; (3) the greatest accumulation of nitrogen in nitrate form at the end of the summer season is always in the well-tilled plots—those of higher moisture content; (4) the accumulation of nitrates at the usual time for fall seeding is the equivalent of very substantial applications of sodium nitrate.

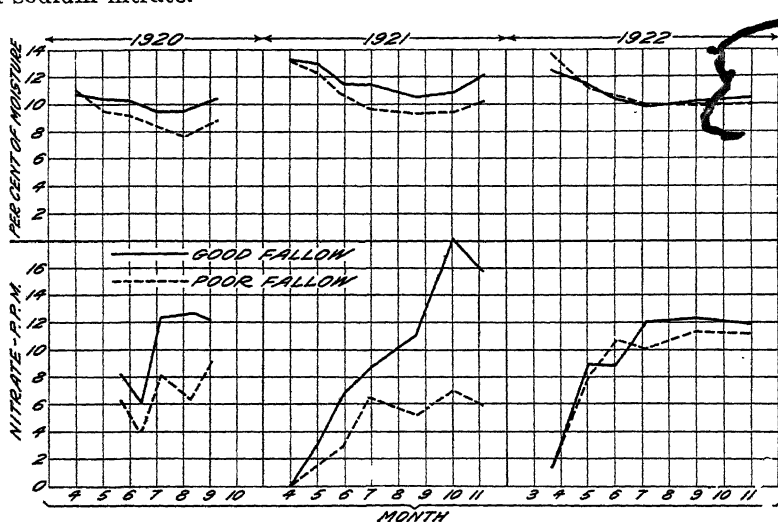


FIG. 5.—Average accumulation of moisture (upper graphs) and of NO_3 (lower graphs), per foot of summer fallow, to a depth of 6 feet, 1920 to 1922, inclusive, in well-tilled and in poorly-tilled plots

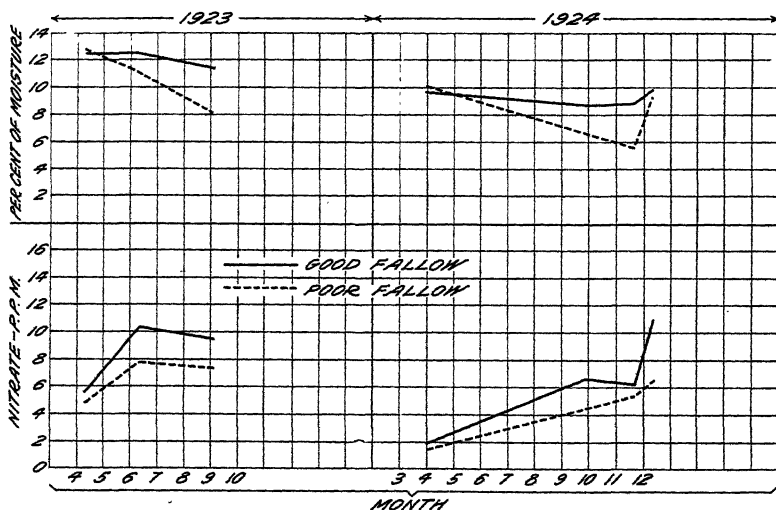


FIG. 6.—Continuation of Figure 5, seasons of 1923 and 1924

It might reasonably be expected that the better tillage treatment increases also the accumulation of other elements of plant food in water-soluble form at seeding time. Only the most delicate methods employed in soil analysis could be made to throw light on that point. The cryoscope seemed to offer the necessary delicacy. In Table 3

are data obtained on the lowering of freezing points by water suspensions of soil from samples taken in three cores to a depth of 24 inches in well-tilled and poorly-tilled plots summer fallowed in 1924. Since (with the exception of samples taken on August 18) the greater

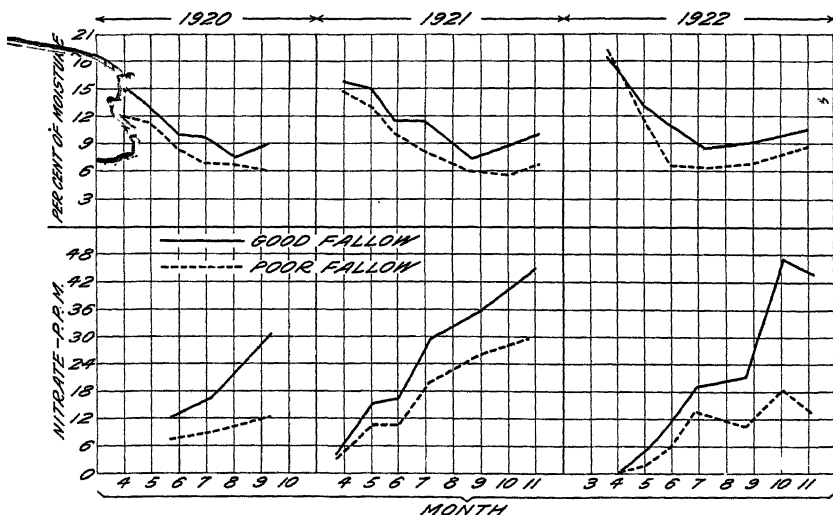


FIG. 7.—Average accumulation of moisture (upper graphs) and of NO_3 (lower graphs), for the first foot of summer fallow, 1920 to 1922, inclusive, in well-tilled and poorly-tilled plots

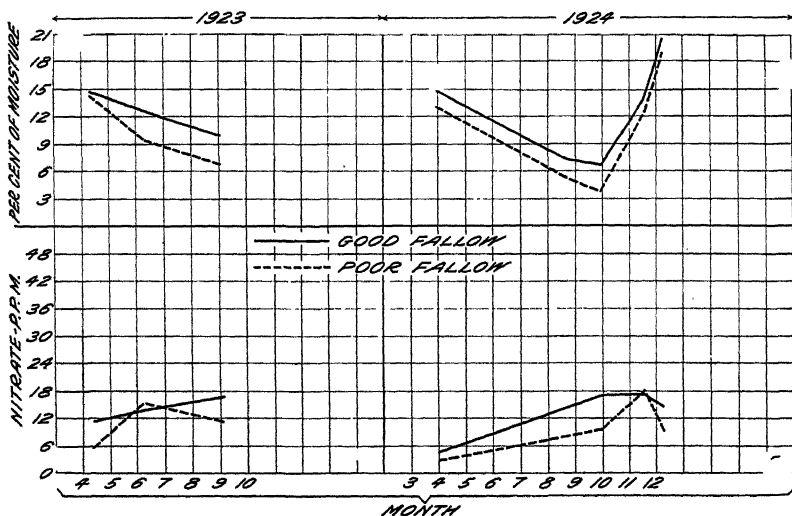


FIG. 8.—Continuation of Figure 7, seasons of 1923 and 1924

depression of the freezing point always occurred with soils from the well-tilled plots, the conclusion that tillage of the summer fallow tends to increase the accumulation of water-soluble plant food appears to be reasonably well founded.

TABLE 3.—*Cryoscopic determinations of water-soluble substance in soils from well-tilled and poorly-tilled fallow plots*

Date of sampling	Depth of core	Depression of freezing point		Date of sampling	Depth of core	Depression of freezing point	
		Well-tilled plots	Poorly-tilled plots			Well-tilled plots	Poorly-tilled plots
1924:	<i>Inches</i>	<i>° C.</i>	<i>° C.</i>	1924—Continued.	<i>Inches</i>	<i>° C.</i>	<i>° C.</i>
July 21.....	0 to 9	0.009	0.007	Oct. 16.....	0 to 9	0.007	0.000
	10 to 18	.002	.000		10 to 18	.002	.002
	19 to 24	.006	.002		19 to 24	.005	.001
Aug. 18.....	0 to 9	.005	.007	Nov. 19.....	0 to 9	.011	.009
	10 to 18	.007	.008		10 to 18	.001	.001
	19 to 24	.002	.000		19 to 24	.005	.000
Sept. 17.....	0 to 9	.009	.005	1925:			
	10 to 18	.025	.010	Apr. 6.....	0 to 9	.012	.008
	19 to 24	.006	.001		10 to 18	.007	.004
					19 to 24	.009	.002

When attempt is made to correlate these facts with the field and laboratory data given in Table 1, difficulties at once arise. Not always do years of greatest yields immediately follow those of greatest nitrate accumulation in the fallowed plots at seeding time. Nor can one with much confidence attribute this fact to differences in moisture retention by the fallowed plots; for, if reference is again made to Figures 3 and 4, it will appear that, notwithstanding the differences in moisture retention during the fallowed season, a remarkable uniformity prevails in the moisture content of all plots at the beginning of the crop season. As a matter of fact, low yields invariably have followed climatic conditions that were unfavorable for germination and emergence of grain in the preceding autumn. The outstanding fact to be noted here is that as between plots of good and poor tillage, grain sown on those of good tillage in the very early stages of growth had a substantial advantage over grain on those of poor tillage in the matter of soil moisture, readily available nitrogen, and perhaps of other readily available plant-food materials as well. As these plots invariably produced the highest yields of grain and straw, which were substantially the richest in protein, the correct inference would seem to be that yellow berry is a manifestation of nutritional disturbance caused by a lack of sufficient nitrogen and perhaps of other essential elements for vigorous plant growth.

IMPORTANCE OF NITROGEN IN DRY-FARMED REGIONS

Simple calculations based upon field and analytical data given in Table 1 put in concrete form two very important facts: (1) Very considerable amounts of nitrogen are being removed annually from all of these plots by the wheat crops they produce; (2) the better the tillage methods practiced on the summer fallow the greater the amounts of nitrogen removed by the succeeding crop.

If the cause of yellow berry in Turkey wheat is correctly surmised from the work reported here, a certain amount of control by summer-fallow tillage appears to be feasible, just so long as soil nitrogen is not too sharply the limiting factor in crop yields. However, one may not reasonably expect control to average well up towards 100 per cent over a long term of years.

The nitrogen problem for dry-farmed lands is perhaps the biggest problem that faces the experiment station at Moro. The removal of nitrogen from the plots at Moro is indicative of what is taking place in the surrounding country, and the question is, how long can the process continue.

SUMMARY

The work reported here is subordinate to a larger project which seeks to determine the influence of variations in summer-fallow tillage on wheat yields.

Thorough tillage of the summer fallow, in contrast to neglect or poor tillage, results in larger yields of grain and straw, both of which are substantially richer in protein and the grain correspondingly less infected with yellow berry.

Since the accumulation of nitrate nitrogen, and seemingly of other water-soluble plant-food substances, at seeding time is always greatest in the well-tilled fallow, it is concluded that yellow berry—low-protein wheat—is the manifestation of nutritional disturbances resulting from insufficiency of nitrogen and other elements of plant food for adequately meeting the requirements of a normally developing crop.

As no means appears feasible as yet for fortifying dry-farmed lands against the gradual depletion of their native supply of nitrogen, it is reasonably certain that high efficiency in the control of yellow berry can not be maintained indefinitely by tillage of the summer fallow, no matter how thorough the tillage may be.

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WESTERN LARCH NURSERY PRACTICE¹

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INTRODUCTION

Although western larch (*Larix occidentalis*) is at present of minor importance in the northern Rocky Mountains and reproduces itself naturally and abundantly on many burned areas, it has not been entirely neglected in planting studies in this region. On lands in need of artificial reforestation there are likely to be some sites better suited to larch than to any other species. To establish larch on these sites it is necessary to produce seedlings or transplants in a nursery, for effective methods of sowing seed directly in the field have not been developed. With this need in view, studies were made to determine the best season for seed sowing, the proper depth to cover seed in seed beds, and the best method of sowing. Two phases of the work of caring for seed beds were also studied, viz, water and shading.

Most of the work was done at a small nursery at Priest River in northern Idaho. Some tests of time and amount of seed for sowing, as well as part of the shade study, were made at Savenac nursery in western Montana. The natural soil at the Priest River nursery, which occupied a gentle southerly slope, was a rather heavy silt, but was made more friable by the addition of fine, sharp sand. The beds, each of which was 48 square feet in size, were protected only with wire screen frames, except in the shade tests, in which lath shade-frames giving half-shade were used. Examination of beds and seedling counts were made at frequent intervals during the periods of rapid germination and less frequently later in the season.

The seed used at Priest River was collected in 1911 on the Colville National Forest in northeastern Washington, and that used at Savenac nursery was collected in 1917 on the Kootenai National Forest in northwestern Montana. Greenhouse tests showed the vitality of the Colville seed to be 15 per cent and that of the Kootenai seed 19 per cent. Had the seed been of better quality the differences in germination resulting from the various nursery treatments probably would have been greater, showing more clearly the relative values of the treatments.

Because good seed and poor seed can not readily be distinguished and separated, the seed from which samples for sowing were to be taken was mechanically mixed in such a way as to distribute the

¹ Received for publication Jan. 25, 1926; issued August. This is a report of work done by D. R. Brewster and E. C. Rogers at Priest River, Idaho, and by the writer at the Savenac Nursery, Haugan, Mont.

It was decided that $\frac{1}{4}$ inch was probably the optimum depth of sand cover, but that in practice it might be well to aim at obtaining $\frac{3}{8}$ inch with an allowable range from $\frac{1}{4}$ inch to $\frac{1}{2}$ inch. Covers shallower than $\frac{1}{4}$ inch obviously are desirable, if the results are satisfactory, but tests have not been made with such covers and nursery practice has been guided by the above experience.

METHOD OF SOWING

The experiment just described, made primarily to study the effect of different thicknesses of sand covering, also afforded a comparison of the two common methods of sowing seed in forest nurseries, broadcast and in drills.

The peak of the germination period occurred on August 5. At that time the drill-sown seeds showed a more rapid rate of response than the broadcast seeds. One way to gauge this rapidity is to express total germination in terms of percentage of the number of seeds sown by each method and compare the figures. The difference in favor of drilled plots varied from 6.7 per cent to 22.5 per cent and averaged 13.6 per cent for six lots; but by August 12 this average difference was reduced to 3.5, and by the end of another week, as the season neared its end, it had almost disappeared.

Total germination is important as well as rapidity of germination. The drilled plots showed larger totals than the broadcast plots in four out of six cases. Each of the two exceptions was a plot with thin sand cover. One was the plot having the least cover applied, and the other was one in which the sand had been washed to one side in sprinkling. With all but the shallowest cover, the total germination of the seed sown in drills tended to be greater than that of the seed sown broadcast, and this tendency was more marked with increase in the depth of cover. A possible explanation of this is that the combined lifting power of numerous seedlings located in a narrow strip was more effective than that of a similar group of seedlings scattered over a larger area. It may be that the lifting and fracture of the cover along the line of the drill allowed a larger number of the naturally weaker seedlings to emerge. The smaller size of western larch seedlings would make them more responsive to minor soil differences of this nature than are pine seedlings.

In the periodic examinations of the plots, most of the losses were attributed to damping-off during August. As resistance to damping-off increases with age and vigor of the seedlings, there would be reason to expect a greater loss in the broadcast plots where germination was less prompt and seedlings of susceptible ages were exposed to infection for a longer period; but, on the contrary, seedling vigor as a whole seems to have been greater in the broadcast plots. This was because fewer of the naturally weaker individuals had emerged from the soil. At the end of the first and second seasons the survival in deeply covered plots was higher from broadcast sowing, while the survival in lightly covered plots was slightly higher from drill sowing.

On the basis of these experiments it was decided, at least where it was not difficult to obtain water for irrigation, that western larch seed should be sown broadcast rather than in drills. While there

is little difference in germination or survival at the desirable shallower depths of sowing, broadcasting tends to eliminate the weakest plants, and it is at the same time the cheaper method. Broadcasting requires less time for soil preparation and sowing; it utilizes the space more completely, and costs less in weeding, watering, and general care.

WATERING SEED BEDS

To obtain information for the guidance of nurserymen as to amounts and intervals for watering, and for rangers and others who might produce small quantities of planting stock in localities where artificial watering would be impossible or very costly, three tests of drill-sown seed were planned: (1) Surface cultivation without artificial watering, (2) surface cultivation with moderate watering, and (3) heavy and frequent watering without cultivation.

On May 6, 1913, 3 beds, each 24 square feet in area, were sown. Drills were spaced 3 inches apart, and were sown with amounts of seed computed to contain 100 good seeds to each foot. Seeds were covered $\frac{1}{4}$ inch deep with sand. Germination was counted once a week in 1913, and once in the fall of 1914.

All beds were watered equally during the period of germination and until August, because the purpose was not to affect germination, but to influence later development. The three test lots thus obtained a fairly even start before August 7, when differentiation in treatment began. For convenience these lots and beds will be referred to as A, B, and C.

Lot A was given no artificial watering after August 7. During the first season it was cultivated after each rain and on every fourth day. During the second season it received the same treatment, except that cultivation between rains was reduced to a frequency just sufficient to maintain a dust mulch.

Lot B was given two quarts of water per square foot every fourth day the first season, and the second season it received enough water artificially applied to make a total of 0.75 inch per week when added to the rainfall for that week. It was cultivated after each rain and watering.

Lot C was not cultivated at any time. It was given one quart of water per square foot every other day the first season, and the second season it was sprinkled enough in addition to rainfall to provide a total of 0.75 inch of water during each half week.

Artificial watering was done on Saturdays and alternate Tuesdays and Wednesdays. When rainfall alone exceeded 0.75 inch during the specified periods for lots B and C, no watering was done, the excess being noted but not carried forward to apply on the next period. Every Monday morning during the second season soil moisture samples were taken from each bed at four depths: Surface inch, 2 to 6 inches, 7 to 12 inches, and 13 to 18 inches. The moisture content in the unwatered, cultivated bed (A) was about 15 per cent lower at all depths than in either of the other two, but differences in moisture between the two artificially watered beds (B and C) were small. The minimum amount of soil moisture, 14 per cent, occurred in bed A; the average moisture content of B and C beds was about 55 per cent, and maxima were near 70 per cent.

Part of the stock was transplanted in November of its second season and the remainder the next April. At the time of fall transplanting 100 plants were mechanically selected from each lot, and lengths of roots, stems, and leaves, and diameters of stems at the ground line, were measured. Averages from these measurements are useful in judging the relative development of plants resulting from different methods of culture. It was found that seedling development was greatest in the bed receiving only cultivation (A), that it was intermediate in the moderately watered bed (B), and was poorest in the bed receiving heavy watering (C). The percentage of trees which survived transplanting was highest, 39 per cent, for the moderately watered stock (B); intermediate, 35 per cent, for the cultivated stock (A); and lowest, 8 per cent, for the heavily watered stock (C). The cultivated stock (A) showed the best development after a year in the transplant bed, with moderately watered (B) and heavily watered stock (C) decidedly poorer.

From these results in drill-sown beds, cultivation alone appeared to be better than either moderate watering with cultivation or heavy watering alone for the production of well developed two-year-old larch seedlings. By drill sowing and cultivation it should be possible in ranger nurseries to produce good larch stock without irrigation. For larger nurseries where seeds are sown broadcast, these experiments indicate that moderate watering is best.

SHADING OF SEED BEDS

On June 6, 1913, and again on May 5, 1914, three plots were sown broadcast to test the effects of one-half and one-quarter shade as compared with full sunlight on seed beds. Enough seed was sown to provide an estimated 300 good seeds per square foot. Counts were made weekly during the period of active germination and biweekly thereafter until the end of the season. In the fall of 1914, samples of stock from all sowings were taken up for measurements of length of roots, stems, and leaves, and diameter of stems at the ground line. Seedlings were transplanted in November, 1914, from each of the plots, with one exception. No trees were moved from the half-shaded plot shown in 1913 because of damage from frost heaving. Survival and development of transplants were recorded in the spring and fall of 1915.

Total germination was greatest under half shade and least under no shade with plots sown in June 1913, but this result was exactly reversed with plots sown in May, 1914. In both years the seedlings started to come up first in unshaded plots, but in the case of the later (June) sowings the shaded plots caught up. This seems to indicate that the seeds are benefited by full sunlight when they begin to sprout, especially early in the season when insolation is less intense, but that when germination is delayed the majority of seedlings will come up only under shade. Of course, survival and development must also be considered.

Because of the inherent slowness of larch seed when spring sown, it would be natural to expect that shade during May and June, even if it made germination still slower, would benefit survival of the

youngest seedlings through its action in reducing transpiration and in keeping the surface soil cooler and more uniformly moist. Shade during these two months, however, appeared to be more detrimental than beneficial because of greater losses from damping-off under the shade frames. Shade on these beds after the 1st of July seemed to benefit survival regardless of whether the seeds sprouted early or late, and half shade was more beneficial than quarter shade. In development during the first year, the trees receiving quarter shade were very slightly larger than the others; after the next year the best developed trees were found in beds having no shade during the second season.

These results were supported by the outcome of some similar work at Savenac nursery. Two 4 by 12 foot beds were sown there on May 18, 1914. One was given no shade at any time, and the other was given half shade from July 1 until the close of the first season. Although the unshaded bed contained more seedlings than its companion on June 22 before either bed was shaded, it fell behind early in July. Periodic seedling counts showed that shade was having a favorable effect upon midseason germination and survival, which by September 8 amounted to a lead of nearly 10 per cent in number of seedlings.

From the results of tests at Priest River and at Savenac nursery, it is recommended that western larch seed beds sown in the spring be given one-half shade during July and August, but no earlier in the first season and none at all during the second.

REMAINING PROBLEMS

In this series of experiments several phases of larch production have not been considered. The proper density in seed beds, the best time for transplanting, and the most suitable age classes deserve attention. With larch, also, some other factor than variation of density in the seed bed is active in producing extremely irregular stands, and this factor is yet to be determined. During their second season, larch seedlings vary roughly from 1 to 12 inches in height. These extremes are greater than in any other species grown in the nursery; a great deal of culling must be done, resulting in a heavy loss in culled plants.

CONCLUSIONS

Larch seeds should be sown in the fall, to avoid hold-over germination, but principally because the first-season germination comes more promptly from seed sown the previous fall.

Present practice aims at $\frac{3}{8}$ inch of sand covering, with allowable variations from $\frac{1}{4}$ to $\frac{1}{2}$ inch. One-quarter inch or less is probably the optimum thickness.

The preferred method of sowing seed is broadcasting rather than sowing in drills. Broadcasting tends to eliminate the weakest plants, with no net loss in survival under the more desirable light sand covering; also the cost is lower in time taken from soil preparation and sowing, in utilization of space, and in the labor demanded for weeding, watering, and general care.

However, where water for irrigation is difficult to obtain, drill sowing may be necessary. It has been shown that well-developed 2-year-old larch seedlings can be produced in the northern Rocky Mountain region in cultivated drills without artificial watering. With broadcast sowing moderate watering is best.

The amount and degree of shade needed by spring-sown seed beds is half shade during July and August, but none earlier than that and no shade at all during the second season.



DISTRIBUTION OF VOLATILE FLAVOR IN GRAPES AND GRAPE JUICES¹

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INTRODUCTION

A knowledge of the distribution of volatile flavor in grapes and grape products is of value to viticulturists who wish to determine botanical origins, to manufacturers of grape juice who are interested in the cause of deterioration of bottled grape juice during storage, and to food officials who regulate the sale of true and imitation grape products.

Methods have not yet been developed for the determination of all the volatile flavoring constituents of grapes, the chemical character of which is still for the most part unknown. Exact quantitative methods for anthranilic acid ester, usually referred to as methyl anthranilate, one of the most important volatile constituents of certain varieties of grapes, however, are now available, and the total esters and total free volatile acids can be readily determined by well-known methods. Power and Chesnut (7)² have shown that the content of anthranilic acid ester in authentic grape juices varies with the variety of grapes. Their data, which were intended to be only approximately quantitative, were expressed in the following terms: ++, strong reaction for methyl anthranilate; +, slight but distinct reaction; - negative result. In a previous publication (6) they stated that the largest quantity of methyl anthranilate (anthranilic acid ester) which they had found in the red juices of the Concord type was about 0.001 gm. per 500 c. c. of grape juice, and that the largest quantity in light-colored juices was not more than 0.0001 gm. per 500 c. c. of grape juice. These quantities are equivalent to 2.0 and 0.2 mgm. per liter respectively. Power and Chesnut also suggested that a determination of the presence or absence of methyl anthranilate may have some diagnostic value. Scott (8), who employed an exact quantitative method, found that eight brands of Concord juice believed to be pure contained from 0.80 to 1.49 mgm. of the ester per liter, and that four brands of Catawba juice believed to be pure contained from 0.11 to 0.40 mgm. per liter.

The purpose of the investigation reported here was to extend the work of Power and Chesnut, and Scott, by determining the exact quantities of anthranilic acid ester, total esters, and total volatile acids in a large number of varieties of grapes and grape juices and by determining the distribution of these constituents in the skins, pulp, and juice of grapes and in the products of the grape resulting from the commercial manufacture of grape juice.

¹ Received for publication Feb. 27, 1926; issued August, 1926.

² Reference is made by number (italic) to "Literature cited," p. 310.

METHODS OF ANALYSIS

Several methods have been proposed for the determination of anthranilic acid ester in essential oils, fruit juices, and commercial flavors. Most of them are based on Erdmann's method, which depends upon diazotization and the formation of an azo dye. Erdmann (3), and Erdmann and Erdmann (2, p. 1216), coupled β -naphthol and also β -naphtholdisulphonic acid with the diazotized anthranilic acid ester. Power (5) tested both β -naphthol and dimethyl-aniline for this purpose, but preferred to use the former because of its greater sensitiveness. Mathewson (4) used sodium- α -naphthol-2-sulphonate for the spectrophotometric estimation of aromatic amino compounds, including anthranilic acid ester. Scott (8) employed alcoholic α -naphthol solution. The writers' present work was begun before the article by Scott³ was published. After some preliminary experiments on the methods then available, the writers chose for study Mathewson's procedure, in which hydrazine sulphate is used to destroy the excess of nitrous acid after diazotization. The method which they finally adopted is applied directly to the steam distillate, thus eliminating the extraction of the distillate and the removal of the solvent by evaporation.

DETERMINATION OF ANTHRANILIC ACID ESTER

APPARATUS

The apparatus used consisted of the following: (1) A steam generator filled with distilled water (an oil can holding 1 gallon will serve the purpose); (2) a Kjeldahl flask, 750 c. c. capacity, with shortened neck, about 10 inches high over all; (3) a spray tube passing through a rubber stopper and reaching to the bottom of the flask; (4) a Kjeldahl distilling tube; (5) a 10 or 12 inch worm condenser, with tube sealed to the outlet, reaching to the bottom of the receiver; (6) an Erlenmeyer flask, 500 c. c. capacity, to be used as a receiver; (7) tinfoil to cover the rubber stoppers to prevent absorption of the esters; (8) a water bath.

REAGENTS

The following reagents were used: (1) N hydrochloric acid; (2) sodium nitrite solution (2 per cent); (3) hydrazine sulphate solution, saturated (about 3 gm. per 100 c. c.); (4) sodium-1-naphthol-2-sulphonate (5 per cent solution); (5) sodium carbonate (25 per cent solution); (6) standard solution of methyl anthranilate containing 1 mgm. of methyl anthranilate per 1 c. c., obtained by dissolving 0.25 gm. of methyl anthranilate in 60 c. c. of 95 per cent alcohol and diluting to 250 c. c.

PROCEDURE

Place enough water in the receiving flask (6) to just cover, or seal, the end of the extended condenser tube. Place the sample in the distilling flask (2). If the volume of sample is less than 200 c. c. add water up to this volume. Connect with the condenser (5) and

³ In this article the statement "3 c. c. of N sodium nitrite," under the heading "Colorimetric Method," 13th line, evidently should be "3 c. c. of N sodium hydroxide."

immerse the flask in the water bath to the level of the contents. When the sample has attained the temperature of the water bath, which should be about boiling, connect with the steam generator and pass a rapid current of steam through the sample for 30 minutes. About 300 c. c. should collect in the receiver during this time. Disconnect the apparatus and wash out the condenser with a little water. Add 25 c. c. of N hydrochloric acid and 2 c. c. of 2 per cent sodium nitrite solution to the distillate. Mix well and allow to stand for exactly 2 minutes. Add 6 c. c. of saturated solution of hydrazine sulphate and mix well for 1 minute, so that the liquid comes in contact with all parts of the flask which may have been touched by the solution when it contained free nitrous acid. Keep the liquid in the flask in rapid motion while quickly adding 5 c. c. of a 5 per cent solution of sodium- α -naphthol-2-sulphonate, and immediately add 15 c. c. of 25 per cent solution of sodium carbonate. The presence of anthranilic acid ester in the distillate is shown by a color ranging from light pink to deep red, the intensity of the color being proportional to the amount of this ester present. The azo dye formed is α -naphtholsulphonic acid diazomethyl anthranilate. Dilute the colored solution to 500 c. c. with distilled water, mix, and compare an aliquot of the solution with a standard or set of standards.

PRECAUTIONS

In diazotizing 88 mgm. of methyl anthranilate, 2 c. c. of 2 per cent sodium nitrite solution will be used. Enough hydrazine sulphate to destroy completely the excess of nitrous acid present must be added. Otherwise sodium nitrosonaphtholsulphonate, which is intensely yellow and modifies the color of the azo dye, is formed. The excess of this reagent must be kept as small as possible, however, in order to limit the number of side reactions.

The proper volumes of the solutions of sodium-1-naphthol-2-sulphonate and of sodium carbonate should be measured in graduated cylinders when beginning the test. This procedure will enable the operator to add the reagents quickly while keeping the solution in rotation, in such a way that each will become incorporated in the liquid almost immediately. About 5 c. c. of 25 per cent sodium carbonate solution is necessary to neutralize the hydrochloric acid present, but the addition of 15 c. c. of this solution gives a solution containing equal parts of sodium carbonate and sodium bicarbonate, which Mathewson found to have the proper hydrogen-ion concentration to give maximum color for this dye. The red dye formed by methyl anthranilate is gradually saponified in alkaline solution, forming the corresponding compound of anthranilic acid, which is more yellow. For this reason it is necessary to apply the test reaction to standard solutions and distillates at as nearly the same time as possible.

Synthetic samples examined by this procedure gave the results reported in Table 1. The methyl anthranilate used in these experiments consisted of two commercial samples, which were shown to contain 97.1 and 97.7 per cent methyl anthranilate, respectively, when analyzed by Mathewson's spectrophotometric method (4).

TABLE 1.—*Anthranilic acid ester in synthetic solutions*

Sample	Ester added	Ester found	Quantity recovered
	<i>Mg</i>	<i>Mg</i>	<i>Per cent</i>
Water.....	1.102	1.086	98.6
Do.....	1.102	1.088	98.8
Do.....	5.51	5.408	98.2
Do.....	5.51	5.463	99.2
Do.....	11.02	10.91	99.0
Do.....	11.02	10.91	99.0
Do.....	27.55	26.94	97.8
Do.....	27.55	26.86	97.5
Grape juice.....	0.00	0.31	-----
Do.....	5.51	5.48	^a 94.2
Do.....	11.02	10.90	^a 96.2
Do.....	27.55	27.48	^a 98.6

^a Based on quantity of ester added, plus quantity found in the commercial sample of grape juice.

The foregoing method has been used for several years with satisfactory results in the field stations of the Bureau of Chemistry of the United States Department of Agriculture for the analysis of genuine and imitation grape products.

DETERMINATION OF TOTAL VOLATILE ACIDS

Subject 200 to 500 c. c. of grape juice or grape beverage to steam distillation, as described in the method for the determination of anthranilic acid ester. Add phenolphthalein indicator to the distillate, and titrate to a permanent pink color with N/10 alcoholic potash. Report as milligrams of acetic acid per liter (1 c. c. N/10 alkali = 6 mgm. acetic acid).

DETERMINATION OF TOTAL VOLATILE ESTERS

After titration of the volatile acids, add 15 to 25 c. c. excess of N/10 alcoholic potash. Heat under a reflux condenser for 1 to 2 hours. Cool and titrate the excess of alkali with N/10 sulphuric acid. Run a blank saponification, using the same quantity of alcoholic potash in 200 c. c. of water made just alkaline to phenolphthalein. Subtract the number of cubic centimeters of acid required for the sample from that required for the blank, and multiply by 8.8. This will give milligrams of ester in terms of ethyl acetate.

MATERIALS

SOURCE OF SAMPLES

The following samples were obtained through the courtesy of J. S. Caldwell of the Bureau of Plant Industry of the United States Department of Agriculture: Whole grapes—32 varieties, 1 sample each; 13 varieties, 2 samples each; 3 varieties, 3 samples each; 1 variety, 4 samples. Grape juice—3 varieties, 3 samples each; 2 varieties, 4 samples each; 1 variety, 5 samples. In most cases each sample of whole grapes came from a single vine. The samples of grape juice had been stored from 1 to 5 years.

Through the courtesy of a manufacturer of grape juice in Chautauqua County, New York, 5 samples of grape juice, representing 1 variety of grapes, and 1 sample of dried pomace, were obtained. This grape juice had been stored from 1 to 3 years.

The following samples were purchased on the Washington, D. C., market in 1924 and 1925: Whole grapes—5 varieties, 1 sample each; 1 variety, 2 samples; 1 variety, 3 samples; 1 variety, 7 samples. Grape juice—8 brands, 1 sample each; 3 brands, 2 samples each.

Altogether 88 samples of whole grapes, representing 55 varieties, and 41 samples of grape juice, representing 6 varieties and 11 brands, were examined.

PREPARATION OF SAMPLES

In the analysis of the whole grapes, 100 to 500 gm. of stemmed grapes were placed in the distilling flask and crushed with a glass rod (flattened at one end) so that all the skins were broken.

"Hot pressed" juice was obtained for analysis by placing weighed quantities of stemmed grapes in a pan, crushing, and heating to boiling. The boiling was continued for three to five minutes, after which the mixture was transferred to a muslin bag and pressed by hand. Two samples were prepared in this way to determine the effect of heating on the content of volatile flavor. "Cold pressed" juice was obtained by placing weighed quantities of stemmed grapes in a muslin bag and pressing with the aid of a screw press. The juices obtained in this way were analyzed without filtering. Other juices were obtained for analysis by draining the pulp and by pressing or draining the skins which had been carefully separated from the pulp by hand. In several instances the juices obtained from draining the pulp and pressing the skins were mixed for analysis. The little juice which drained off in separating the skins from the pulp was collected and analyzed separately. The pomace, pressed or dried skins, and drained pulp, were analyzed, as was also a sample of commercial dried pomace. The samples of authentic and commercial grape juices, of course, required no special preparation for analysis.

EXPERIMENTAL RESULTS

The quantities of anthranilic acid ester, volatile esters, and volatile acids in 84 samples of whole grapes, representing about 55 varieties, are given in Table 2. Sixty samples, representing about 41 varieties, did not contain a measurable quantity of anthranilic acid ester. The quantities of anthranilic acid ester in the other samples varied from 0.05 to 3.80 mgm. per kilogram. The samples which contained the larger quantities of anthranilic acid ester were more fragrant and more highly flavored than the others, except two samples of the Campbell variety, which contained no anthranilic acid ester but did contain exceptionally large quantities of other volatile esters. The volatile esters appeared to vary directly with the fragrance and flavor of the grapes. The three varieties of the *vinifera* species (Tokay, Cornocho, and Malaga) did not contain anthranilic acid ester. Power and Chestnut (?), who examined two comparatively fresh samples of pasteurized grape juice of this species (Muscat and Petite Sirah), found no anthranilic acid ester. Five additional samples of juice of this species (Burger, Sauvignonvert, Zinfandel, Alicante, and Bouschet) examined by them also gave no reaction for anthranilic acid ester. These data indicate that grapes of the *Vitis vinifera* species do not contain anthranilic acid ester. The one sample of Cunningham variety of whole grapes (*Vitis bourquiniana*) which

was examined contained 0.10 mgm. of anthranilic acid ester per kilogram. Power and Chestnut (?) reported negative results on a sample of grape juice of the species *V. bourquiniana*, variety Herbemont.

TABLE 2.—Flavor in authentic and commercial samples of whole grapes, 1924 and 1925

Variety and species ^a	Anthranilic acid ester ^b	Volatile esters ^c	Volatile acids ^d	Anthranilic acid ester in volatile esters ^e	Variety and species ^a	Anthranilic acid ester ^b	Volatile esters ^c	Volatile acids ^d	Anthranilic acid ester in volatile esters ^e
	Milligrams per kilogram			Per cent		Milligrams per kilogram			Per cent
Lutie (L).....	0.00	28	37	0.0	Tokay ^a (V).....	0.00	-----	-----	0.0
Do.....	.00	141	35	.0	Cornochon ^a (V).....	.00	-----	-----	.0
Alice (L).....	.00	57	35	.0	Malaga ^a (V).....	.00	-----	-----	.0
Do.....	.00	-----	-----	.0	Diogenes (RL).....	.00	-----	-----	.0
Vergennes (L).....	.00	6	3	.0	Do.....	.00	-----	-----	.0
Bertha (L).....	.00	84	84	.0	Franklin (RL).....	.00	-----	-----	.0
Early Victor (LB).....	.00	38	62	.0	Do.....	.00	46	-----	.0
Valhalla (LCRVB).....	.00	-----	-----	.0	Stray.....	.00	-----	-----	.0
Requa (LV).....	.00	-----	-----	.0	Louisiana (B).....	.00	8	35	.0
Do.....	.00	-----	-----	.0	Do.....	.00	14	46	.0
Cuyahoga (LV).....	.00	-----	-----	.0	Canada (LV).....	.00	14	23	.0
Lindley (LV).....	.00	-----	-----	.0	Stray.....	.00	39	16	.0
Jefferson (LV).....	.00	-----	-----	.0	Do.....	.00	113	46	.0
Kingessing (LV).....	.00	-----	-----	.0	Do.....	.00	15	63	.0
Iona (LV).....	.00	-----	-----	.0	Marguerite (LiB).....	.00	-----	-----	.0
Do.....	.00	51	39	.0	Big Hope (LiLV).....	.00	174	121	.0
Do.....	.00	156	59	.0	Cloeta (LiRuLV).....	.00	20	48	.0
Do.....	.00	15	16	.0	Rogers No. 32 (LV).....	.00	-----	-----	.0
Campbell (LV).....	.00	360	70	.0	Do.....	.00	37	59	.0
Do.....	.00	366	52	.0	Hartford (LV).....	.05	-----	-----	.0
Rebecca (LV).....	.00	51	30	.0	Arkansas (L).....	.07	-----	-----	.0
Do.....	.00	-----	-----	.0	Cunningham (B).....	.10	-----	-----	.0
Lightfoot (LV).....	.00	71	29	.0	Salem ^a (LV).....	.12	19	61	.4
Essex (LV).....	.00	16	3	.0	Brighton ^a (LV).....	.12	64	71	.1
Merrimac (LV).....	.00	52	33	.0	Etta (RL).....	.14	-----	-----	.0
Mills (LV).....	.00	17	8	.0	Rogers No. 32 (LV).....	.27	29	35	.5
Do.....	.00	-----	-----	.0	Cottage (L).....	.28	188	62	.1
Salem (LV).....	.00	-----	-----	.0	Do.....	.30	192	53	.1
Diana (LVA).....	.00	48	48	.0	Loretto (L).....	.30	130	40	.1
Do.....	.00	51	27	.0	Delaware ^a (LBV).....	.36	-----	-----	.0
Brilliant (LVB).....	.00	9	67	.0	Do.....	.50	-----	-----	.0
Do.....	.00	17	51	.0	Pearl (RL).....	.90	6	22	8.8
Brilliant (seed) (LVB).....	.00	16	37	.0	Concord (L).....	.91	-----	-----	.0
Bailey (LiLV).....	.00	-----	-----	.0	Pearl ^a (RL).....	.95	7	23	7.9
Mary Favorite (RL).....	.00	39	47	.0	Concord ^a (L).....	1.39	57	9	1.4
Do.....	.00	42	28	.0	Do.....	1.40	103	53	.8
Do.....	.00	50	42	.0	Fern Munson (LiLV).....	1.40	-----	-----	.0
Clinton (RL).....	.00	8	7	.0	Concord ^a (L).....	1.70	-----	-----	.0
Do.....	.00	8	5	.0	Niagara ^a (LV).....	2.72	46	7	3.4
Gaertner (VL).....	.00	-----	-----	.0	Concord ^a (L).....	3.50	170	50	1.2
Goethe (VL).....	.00	6	37	.0	Stray.....	3.75	-----	-----	.0
					Concord ^a (L).....	3.80	170	50	1.3

^a L, labrusca; B, bourquiniana; C, candicans; R, riparia; V, vinifera; A, aestivalis; Li, linccumli; Ru, rupestris.

^b Expressed as methyl anthranilate.

^c Expressed as ethyl acetate.

^d Expressed as acetic acid.

^e Anthranilic acid ester calculated to ethyl acetate.

^f Analysis made on 100 to 400-gm. samples; all others on 500-gm. sample.

^g Commercial samples represented to be of the variety and species indicated; all others were authentic.

Table 3 shows the distribution of flavor as represented by anthranilic acid ester and total esters in different parts of grapes. It

has been generally thought that the volatile flavor of grapes resides almost wholly in the skins. This is not the case, however, as 32, 29, 38, and 44 per cent of anthranilic acid ester, and 56 and 51 per cent of total volatile esters, were found in the drained pulp. It is interesting to note also that one-half or more of the total anthranilic acid ester, and more than one-third of the total volatile esters, remain in the pomace, to be lost unless the pomace is worked over for "second pressing" juice. The sample of commercial pomace which had been dried contained a large quantity of flavor. The largest quantity of anthranilic acid ester which was found was 19.5 milligrams per kilogram of pressed skins of the Concord variety of grapes.

TABLE 3.—*Distribution of flavor in different parts of the grape*

Variety ^a and species ^b	Preparation of sample	Anthranilic acid ester ^c	Volatile esters ^d	Volatile acids ^e	Anthranilic acid ester in volatile esters	Juice, skin, or pulp ^f	Anthranilic acid ester ^g	Volatile esters ^h	Volatile acids ⁱ
		Mg. per liter	Mg. per liter	Mg. per liter	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
Delaware (LBV)...	Juice:								
	Pressed from whole grapes (hot).	0.15	70	22	0.1	75	24	57	32
Do.....	Pressed from whole grapes (cold).	.15	21	18	.4	88	24	64	13
Do.....	Pressed from skins and drained from pulp.	.18	-----	-----	-----	34	18	-----	-----
Niagara (LV).....	Pressed from whole grapes (hot).	2.50	11	26	13.3	85	44	-----	-----
Do.....	Pressed from whole grapes (cold).	2.08	20	11	6.0	90	40	-----	-----
Do.....	Pressed from skins and drained from pulp.	2.33	-----	-----	-----	46	48	-----	-----
Concord (L).....	Pressed from skins.....	4.4	128	53	2.0	33	37	32	30
Do.....	Drained from pulp.....	1.6	75	9	1.3	15	6	8	23
Do.....	Drained from skins and pulp.	1.7	83	32	1.2	31	16	22	17
	Pressed skins, pulp, and seeds:								
Delaware (LBV)...	Hot.....	^ 47	^ 53	^ 46	.5	25	76	43	68
Do.....	Cold.....	^ 47	^ 12	^ 119	2.3	12	76	36	87
Niagara (LV).....	Hot.....	^ 3.23	-----	-----	-----	15	56	-----	-----
Do.....	Cold.....	^ 3.07	-----	-----	-----	10	60	-----	-----
Delaware (LBV)...	Pressed skins.....	^ 2.15	-----	-----	-----	8	23	-----	-----
Niagara (LV).....	do.....	^ 13.1	-----	-----	-----	4	19	4	4
Concord (L).....	do.....	^ 19.5	^ 127	^ 58	9.0	4	19	4	4
Do.....	Drained skins.....	^ 6.0	^ 149	^ 126	2.4	21	40	27	46
Delaware (LBV)...	Drained pulp.....	^ 2.20	-----	-----	-----	55	32	-----	-----
Niagara (LV).....	do.....	^ 1.43	-----	-----	-----	44	29	-----	-----
Concord (L).....	do.....	^ 3.0	^ 154	^ 49	1.1	48	38	5b	43
Do.....	do.....	^ 3.0	^ 129	^ 48	1.3	47	44	51	37
Do.....	Commercial dried pomace.....	^ 16.0	^ 69	^ 28	13.4	-----	-----	-----	-----

^a Commercial samples represented as being of the variety indicated.

^b L, labrusca; B, bourquiniana; V, vinifera.

^c Expressed as methyl anthranilate.

^d Expressed as ethyl acetate.

^e Expressed as acetic acid.

^f Quantity based on weight of whole grapes.

^g Based on total quantities in whole grapes.

^h Milligrams per kilogram.

Data showing change of flavor in grape juice during storage are set forth in Table 4. These data indicate that the content of anthranilic acid ester varies inversely with the period of storage. This is especially noticeable in the case of the Niagara grape juice, no anthranilic acid ester having been found in the sample which had

been stored 5 years, whereas a sample of fresh juice contained 2.08 mgm. of anthranilic acid ester per liter. The low figures for Concord grapes in 1924 may be due to a number of factors. The volatile flavor in grapes is probably subject to seasonal variation, although no data definitely prove it. Caldwell (1) has shown that climatic conditions, especially the amount of sunshine during the growing season, markedly affect the total sugar content, total astringency, and titratable acidity of grapes. The fact that the grape-growing season of 1924 in the eastern part of the United States had less sunshine than that of 1919, 1920, 1921, 1922, 1923, and 1925 may explain the low figures reported.

TABLE 4.—*Change in flavor of grape juice during storage*

Variety and species ^a	Crop	Years in storage	Anthranilic acid ester ^b	Volatile esters ^c	Volatile acids ^d
			Milli-grams per liter	Milli-grams per liter	Milli-grams per liter
Agawam (LV).....	1923	1	0.00	-----	-----
Do.....	1921	3	.00	-----	-----
Do.....	1920	4	.00	-----	-----
Do.....	1919	5	.00	-----	-----
Catawba (LV).....	1923	1	.03	-----	-----
Do.....	1921	3	.00	-----	-----
Do.....	1919	5	.00	-----	-----
Concord * (L).....	1925	-----	.80	32	13
Do.....	1924	1	.20	26	10
Do.....	1924	1	.24	21	8
Do.....	1923	1	.50	-----	-----
Do.....	1923	2	.76	39	16
Do.....	1922	3	.70	37	15
Do.....	1921	3	.17	-----	-----
Do.....	1920	4	.20	-----	-----
Do.....	1919	5	.06	-----	-----
Delaware (LBV).....	1924	-----	.15	-----	-----
Do.....	1923	1	.00	-----	-----
Do.....	1920	4	.00	-----	-----
Do.....	1919	5	.00	-----	-----
Ives (LA).....	1923	1	1.10	-----	-----
Do.....	1920	4	.16	-----	-----
Do.....	1919	5	.07	-----	-----
Niagara (LV).....	1924	-----	2.08	-----	-----
Do.....	1923	1	.68	-----	-----
Do.....	1922	2	.55	-----	-----
Do.....	1921	3	.18	-----	-----
Do.....	1920	4	.12	-----	-----
Do.....	1919	5	.00	-----	-----

^a L, labrusca; V, vinifera; B, bourquiniana; A, aestivis.

^b Expressed as methyl anthranilate.

^c Expressed as ethyl acetate.

^d Expressed as acetic acid.

* Commercial samples believed to be of the variety indicated. Pressed hot, bottled, and pasteurized. All other samples were authentic, and were pressed cold, bottled, and pasteurized.

The following experiment was conducted to supplement the data which indicated a loss of volatile flavor during storage. A sample of commercial grape juice examined on July 15, 1924, contained 1.35 mgm. of anthranilic acid ester per liter. To this sample was added methyl anthranilate in the proportion of 5.21 mgm. per liter. The sample, which then contained 6.56 mgm. of anthranilic acid ester per liter, was stored for about 10 months. On May 26, 1925, it contained 4.05 mgm. of anthranilic acid ester per liter, a decrease of

2.51 mgm. per liter. It was then stored for a further period of 8 months, at the end of which time (January 29, 1926) it contained only 0.70 mgm. of anthranilic acid ester per liter. The data on volatile esters in Table 4 are not sufficient to warrant any conclusions.

Data on the flavor in 14 samples of commercial bottled grape juices are given in Table 5. The anthranilic acid ester varied from 0.00 to 1.35 mgm. per liter in these samples. The volatile esters in 8 of the samples varied from 5 to 29 mgm. per liter.

TABLE 5.—*Flavor in 14 samples of commercial grape juice*

Sample	Variety and species ^a	Anthra- nilic acid ester ^b	Volatile esters ^c	Volatile acids ^d	Anthra- nilic acid ester in total volatile esters ^e
		Milli- grams per liter	Milli- grams per liter	Milli- grams per liter	Per cent
A.....	?	0.00			0.0
B.....	Catawba / LV	.00			.0
C.....	Catawba / LV	.00			.0
D.....	Concord / L	.16	17	7	.6
E.....	Concord / L	.16			
E.....	Concord / L	.20	5	70	2.3
F.....	Concord / L	.25	5	37	2.9
G.....	Concord / L	.35	8	43	2.5
H.....	?	.35	5	69	4.1
I.....	?	.40	5	99	4.7
J.....	?	.45	5	97	5.2
H.....	?	.56			
K.....	Concord / L	.85	29	12	1.7
G.....	Concord / L	1.35			

^a L, labrusca; V, vinifera.

^b Expressed as methyl anthranilate.

^c Expressed as ethyl acetate.

^d Expressed as acetic acid.

^e Anthranilic acid ester calculated to ethyl acetate.

^f Variety obtained from the bottle labels and therefore not authentic.

CONCLUSIONS

Anthranilic acid ester in grapes, grape products, and imitation grape preparations can be determined quickly and accurately by a new method developed in the Bureau of Chemistry of the United States Department of Agriculture.

The content of anthranilic acid ester, and of total volatile esters is a true measure of the intensity of aroma in grapes and grape juices.

The anthranilic acid ester in 84 samples of whole grapes, representing about 55 varieties, varied from 0.00 to 3.80 mgm. per kilogram. The volatile esters and volatile acids in 50 samples, representing about 34 varieties, varied from 6 to 366 and from 3 to 121 mgm. per kilogram, respectively.

Anthranilic acid ester has not been found in the fruit of *Vitis vinifera*. The determination of this ester, therefore, appears to be of value in identifying species.

Contrary to general opinion, the volatile flavor of grapes is not contained almost wholly in the skins. Substantial proportions are found in the pulp. Nearly as much volatile flavor remains in the pomace as is contained in the expressed juices.

Anthranilic acid ester tends to disappear from grape juice which is stored. This fact may explain the deterioration in flavor of certain commercial bottled grape juices.

The anthranilic acid ester in 14 samples of commercial bottled grape juices of unknown origin varied from 0.00 to 1.35 mgm. per liter. The volatile esters in 8 of these samples varied from 5 to 29 mgm. per liter.

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man (fig. 2, G, H, N) spicules with bluntly rounded tips were found. Similar spicule tips were found in certain specimens from swine (fig. 2, I). Some specimens from swine had gradually tapering spicule tips which terminated in sharp points (fig. 2, O). In one specimen from a pig (fig. 2, K) a spicule tip very similar to that figured by Schneider for the human *Trichuris* (fig. 2, L) was found.

It is evident from these observations that the shape of the spicule tip is variable in *Trichuris* from man, monkeys, and swine, and that there is no relation between the shape of the spicule tip and the host from which the worm was obtained, practically the same range of variation occurring in all hosts.

The shape of the sheath presented a similar range of variation, as may be seen by comparing G, H, I, and N of Figure 2, which show practically the same type of sheath in specimens from man, the chimpanzee, and the pig as that figured by Schneider (fig. 2, L) (8) as characteristic of the sheath in the human *Trichuris*. J, M, and Q of Figure 2 show cylindrical sheaths in specimens of *Trichuris* from the pig, man, and *Cercopithecus*, respectively. Similar cylindrical sheaths from the human *Trichuris* have been figured by Goeze (fig. 1, A) (3) and by Urioste (fig. 1, F) (10). Davaine's figure (2) of the spicule sheath of *Trichuris* from man (fig. 1, E) is very similar in shape to the figure given by Schneider as characteristic of *Trichuris* from the pig (fig. 2, P).

As far as the abundance and shape of the spines are concerned, the present writer found specimens from the chimpanzee in which the spines were practically absent in the posterior region of the sheath (fig. 2, G, H), a character which Schneider considered specific for the swine whipworm. In a specimen of *Trichuris* from man (fig. 2, N) the pointed spines in the posterior region of the worm are replaced by blunt scaly spines, whereas in most specimens of swine whipworms examined the entire sheath was thickly covered with pointed spines, the latter a character considered by Schneider as specific to *Trichuris* from man.

On the basis of these observations it appears that neither the shape of the sheath, nor the abundance and shape of the spines on the posterior part of the sheath, nor the character of the tip of the spicule is of specific value in differentiating *Trichuris* from man, monkeys, and swine, because the same degree of variation with respect to these characters occurred in the parasites examined from all the hosts mentioned. The spicule sheath in *Trichuris* is a retractile organ and apparently capable of considerable expansion and contraction, since the sheath in retracted position is much narrower than when extruded. The abundance and shape of spines may possibly be correlated with the age or with other conditions of different specimens, or these characters are normally exceedingly variable. The observations recorded in this paper are in harmony with the discrepancies in shape of the different figures of the posterior end of the male of *Trichuris* from man and from swine, and they support the doubts expressed by Creplin (1) and Leuckart (4) concerning the validity of *T. crenatus* as a species distinct from *T. dispar* (= *T. trichiura*), and they lead the present writer to the conclusion that as far as morphological organization is concerned the swine whipworm belongs to the same species as that occurring in man and in certain other primates. The name *Trichuris suis* must therefore be considered a synonym of *T. trichiura*.

In view of the medical significance of the conclusion that the swine and human whipworms are zoologically indistinguishable, it is highly important to determine whether these worms have developed specific host adaptations, or whether the embryonated eggs of the worms from one host species will be infectious for the other host species. While experiments on man are practically precluded, or at least of doubtful advisability, experiments involving feeding embryonated eggs of human whipworms to swine and of embryonated eggs of swine whipworms to monkeys will be undertaken as soon as the necessary material has been successfully cultured.

SUMMARY

A morphological comparison of whipworms (*Trichuris*) from man, the chimpanzee, *Cercopithecus*, and swine, has shown that the worms from these hosts are morphologically indistinguishable, as far as they have been compared. Schneider's differentiation between whipworms from man and other primates and those occurring in swine have been shown to be due to individual variation, since the characters regarded by Schneider as specific for whipworms from primates are present in whipworms from swine, and vice versa. *Trichuris suis* and its synonyms (*T. crenatus* and *T. apri*) must for the present be regarded as synonyms of *Trichuris trichiura*.

Since the probability that the human and swine whipworms are identical implies the possible necessity for taking cognizance of swine as sources of infestation for man, it is important that the possible transmission of *Trichuris* from man to swine and from swine to man, or at least to other primates, be investigated experimentally, and it is hoped to carry out such experiments at an early date.

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LABORATORY EXPERIMENTS WITH ARSENICALS IN THE CONTROL OF THE CODLING MOTH¹

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INTRODUCTION

It has been the experience of investigators in codling-moth control that orchard-plot tests of various spray materials are not always dependable. Where the difference in results is expected to be small, owing to the variation in the treatment, the difference is often entirely obscured by factors over which the investigator has little or no control. It is well known that natural variations in infestation occur even in small orchards. These may be due to different varieties, to the presence of an adjacent badly infested orchard, or to a fruit, packing house. But variations often occur where they would not be expected, and where they can not be forecast.

Several methods have been suggested to overcome these variations. It is possible to spray the whole experimental plot alike the first year, and determine the variation by examining the fruit from a number of trees suitably distributed over the plot. Ball (1)² recognized the fact that variations occurred, and employed a method of comparing the sprayed trees with adjacent unsprayed trees, giving his results in terms of efficiency rather than in terms of percentage of wormy fruit. Felt (5) mentioned a possible relation of the number of "stings" to the total number of wormy apples, and Melander (10) suggested employing the ratio of worms to stings to show the results from different treatments. The use of more modern statistical methods may also help to eliminate some of the errors due to natural variations.

Laboratory tests, consisting of feeding sprayed foliage to insects or their larvae, have been conducted by various investigators, notably Tartar and Wilson (21), Scott and Siegler (16), Lovett (8), and Cook and McIndoo (3). This method does not seem to have been employed with the codling moth,³ and it seemed to the writer that many of the uncontrollable factors met with in codling-moth work could be avoided by its use. Accordingly, the writer has made a large number of laboratory tests, consisting essentially of spraying a given number of apples, placing a given number of newly hatched codling-moth larvae on them, and later recording the number of worm holes and stings. This method can be used to show the relative value of various arsenicals, of various dilutions of arsenicals, and of combinations of arsenicals with other spray materials. The ease with which it can be employed enables the investigator to carry out a large number of tests in a single season and to make direct compari-

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² Reference is made by number (italic) to "Literature cited," p. 329.

³ Ralph H. Smith has made a number of tests of this nature, and has presented some of the results in a paper entitled "Experiments on the Efficiency of Lead Arsenate in Protecting Apples against Codling Moth Injury." This paper was read before the meeting of the Pacific Slope Branch of the American Association of Economic Entomologists June 27, 1924.

sons of a relatively large number of variations in the treatment. Obviously, this method gives only an index of the value of the material used at the time it is applied. It is of no value in determining the durability or adhesiveness of the arsenicals, or of the relative effect of various methods of application (such as by means of the spray gun or rod), or of various numbers of applications, though it has been used to show the difference between light and heavy applications of spray.

The writer has made nearly 300 tests of this nature in 1922, 1923, and 1924, at the fruit-insect investigations laboratory of the United States Bureau of Entomology at Yakima, Wash.

METHODS

After some experimenting, the following method, designed to eliminate experimental error as far as possible, was employed. Only unsprayed apples free from worms were used. Jonathans were used for all of the tests in 1923; Arkansas Blacks were used in 1924, they being available in larger quantities. The calyx was cut from each apple and the wound was filled with melted paraffin, which prevented worms from entering the calyx. The apples were then suspended from strings by their stems in an outdoor shelter. A sticky tree-banding material was put around the strings to prevent stray worms from crawling down to the apples, since large numbers of worms were being reared in the shelter.

The spray was applied with a small hand sprayer, the apples being rotated to insure an even coating. When the apples were thoroughly dry, newly hatched larvae were carefully placed on them by means of a camel's-hair brush. These larvae were obtained from jars in which moths had deposited large numbers of eggs, and they were transferred to the apples as fast as they hatched. Care was taken to use only fresh, active larvae. The larvae available from any one jar were distributed as evenly as possible on all the apples used, in case there should be a difference in the vitality of different lots of larvae. Usually all were placed within 36 hours of applying the spray.

In 1923 three apples were used for each experiment, 50 larvae being placed on the three apples, 16 on one of them and 17 on each of the other two. In 1924 five apples were used and 10 larvae were placed on each. Usually 10 or 12 tests were made at the same time, materials that were to be directly compared being tested. For example, the experiments made one day would include only different strengths of lead arsenate, whereas those made another day would include different arsenicals, or arsenicals with and without other materials. Each test was repeated several times.

In all of the tests of a given material for each year, material from the same container was used, and only one brand of material was used for all of the tests, except where tests of different brands were made. The lead arsenate used in all of the tests was acid lead arsenate.

The worm entrance holes and stings were counted 10 days after the last larvae had been placed on the apples. Blemishes extending not more than one-fourth of an inch into the flesh were considered as stings. A few of the larvae may have produced both a sting and a worm hole, but for the most part the dead larva could be found in the sting, while in the case of the wormhole the larva was still alive and feeding.

RESULTS OF TESTS

In 1922 the tests were of a preliminary nature, and were of value chiefly in determining the best method to employ. The 1923 tests (all made with second-brood larvae) gave some good results, although, owing to a scarcity of larvae part of the time, it was not always possible to make the desired comparisons on the same date. Comparative tests not made on the same date have been eliminated from the tables, as there is danger of error due to differences in the maturity of the apples and to possible differences in the vitality of the larvae.

In 1924 plenty of larvae were available, and it was possible to make the comparative tests on the same dates. For the most part, two tests were made with first-brood larvae, and one test with second-brood larvae.

In presenting the results of these tests in the tables, it has been necessary to give only the summary of all the tests made with any given material or combination of materials. The total number of worms used is not shown, since in each case it is equal to 50 times the number of tests made. The percentage of worms producing wormholes and the percentage producing stings are both recorded.

The more effective treatment is one that not only reduces the number of larvae producing wormholes or total blemishes, but also causes more of the larvae that do produce blemishes to die after producing only a sting. In other words, the more effective treatment is accompanied by more stings than the less effective treatment, but the reduction in the number of wormholes is relatively greater. This production of stings appears to be unavoidable, because rather slow-acting poisons must be used to avoid injury to the fruit or foliage. Fortunately, stung apples are usually worth four or five times as much as wormy apples, though not as much as entirely sound fruit. It was on account of this increase in the relative number of stings with the more effective treatments that Melander (10) suggested using the ratio of worms to stings in presenting results of spraying tests. For the same reason the number of stings per wormhole is shown in the tables in this paper, as well as the percentage of larvae producing wormholes and stings. This ratio of stings to wormholes is, as a rule, greater in the more effective than in the less effective treatments. In all of the tests, comparison is made with powdered lead arsenate, diluted to 1 pound to 50 gallons of water, this being considered the standard treatment.

CASEIN SPREADER

Very little has been published to show whether the addition of casein spreaders to insecticides increases their effectiveness. Lovett (8), in orchard tests against the codling moth in 1918 and 1919, obtained approximately equal results when he used half-strength lead arsenate with casein spreader, and full-strength lead arsenate without spreader. Stearns and Hough (19, 20), using the standard quantity of lead arsenate, with and without casein spreader, reported no difference in results against the codling moth and other biting insects. Newcomer (11) obtained slightly better control of the codling moth with spreader added to the lead arsenate than without. No laboratory tests appear to have been reported.

In 1923 the present writer made a number of laboratory tests with the codling moth, using a prepared casein spreader containing approximately 20 per cent of casein and 80 per cent of hydrated lime. This was added to the standard dilution of powdered lead arsenate at the rate of one-fourth pound and one-half pound to 50 gallons. A test was also made with three applications of the latter strength sprayed on within a few minutes of each other. It was thought that a thicker coat of spray might be obtained in this way. These tests are reported in Table 1, and show a decided improvement in control where the spreader was added. In test 2, with one-fourth pound of spreader, only a few more than half as many larvae entered the fruit as with the standard treatment in test 1, and the number of stings was slightly less. In test 3, with double the amount of spreader, the results were not as good as in test 2, but were better than in test 1. Test 4, sprayed three times, gave approximately the same results as test 3.

In 1924 these tests were repeated, and spreader at the rate of one-eighth pound to 50 gallons was also used. The results reported in Table 2 show that this quantity (test 2) was the best of those tried, and the number of worms entering the fruit was progressively greater as the amount of spreader was increased (tests 3 and 5). In test 4, sprayed three times, noticeably better results were obtained than in test 3, which was sprayed once. All of the tests with spreader gave better results than where no spreader was used.

TABLE 1.—Comparison of effectiveness of lead arsenate when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1923

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate, 1-50; no spreader.....	4	47	23.5	47	23.5	47.0	1.00
2	Lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50.....	4	25	12.5	35	17.5	30.0	1.40
3	Lead arsenate, 1-50; casein spreader, $\frac{1}{2}$ -50.....	4	28	14.0	53	26.5	40.5	1.89
4	Lead arsenate, 1-50; casein spreader, $\frac{1}{2}$ -50; sprayed 3 times.....	4	30	15.0	47	23.5	38.5	1.57
5	Control (unsprayed).....	4	125	62.5	15	7.5	70.0	.12

TABLE 2.—Comparison of effectiveness of lead arsenate when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1924

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate, 1-50; no spreader.....	3	40	26.7	34	22.7	49.4	0.85
2	Lead arsenate, 1-50; casein spreader, $\frac{1}{8}$ -50.....	3	22	14.7	30	20.0	34.7	1.36
3	Lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	28	18.7	26	17.3	36.0	.93
4	Lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50; sprayed 3 times.....	3	20	13.3	20	13.3	26.6	1.00
5	Lead arsenate, 1-50; casein spreader, $\frac{1}{2}$ -50.....	3	37	24.7	24	16.0	40.7	.65
6	Control (unsprayed).....	3	83	55.3	6	4.0	59.3	.07

Casein spreader was also added to various strengths of lead arsenate and to other arsenicals and spray combinations. The results are noted in the discussions of the respective experiments. The results obtained where casein spreader was used were better than where it was not used, with one exception (tests 8 and 11, Table 11), but in this case it is possible that a reaction occurred between the lime in the spreader and the calcium arsenate, which affected the results.

The addition of casein spreader to the lead arsenate gave a greater improvement in control in these laboratory tests than has been obtained in orchard tests. This suggests that, when freshly applied, the coating of poison with the spreader is much more effective than that without, this being due perhaps to greater uniformity of the coating; but that the coating with spreader is not as durable, due possibly to its being thinner and thus being rendered ineffective more rapidly by the growth of the fruit or by being rubbed off.

After the spreader was added to the spray, better spreading was accomplished when the spray was allowed to stand for about five minutes before using than if it was used immediately.

STRENGTH OF LEAD ARSENATE

Lead arsenate is ordinarily used against the codling moth at the rate of 2 pounds of paste or 1 pound of powder to 50 gallons of water, and this amount has become the standard recommendation. E. D. and W. M. Ball (1) obtained an efficiency of 90 per cent with paste lead arsenate used at the rate of $2\frac{1}{2}$ pounds to 50 gallons, and only 80 per cent efficiency with the same material used at the rate of $1\frac{1}{4}$ pounds to 50 gallons. These tests were made under rather wormy conditions. Melander (9), working under favorable conditions, reported very little difference with paste lead arsenate at various strengths from 1-12 to 1-80. De Sellem (4) obtained better results with the paste lead arsenate at 2-50 than at 1-50, and equal results with powdered lead arsenate at 1-50 and at $\frac{1}{2}$ -50, with a rather light infestation of worms; and Siegler and Plank (18), working in a very wormy orchard, apparently obtained better results with powdered lead arsenate at $\frac{1}{2}$ -50 than at 1-50. A comparison of the total number of stings with the total number of worms in the latter case, however, is in favor of the larger quantity of lead arsenate. All of these tests were orchard tests, and there is a probability that other factors influenced the results.

Lovett (8), in laboratory tests with tent caterpillars, obtained more rapid killing as the strength of paste lead arsenate was increased from $\frac{1}{8}$ -50 to 2-50, and chemical analyses showed more arsenic oxide (As_2O_5) in the bodies of the caterpillars as the strength was increased.

The present writer made a number of tests with the codling moth, using powdered lead arsenate at various strengths from one-half pound to 3 pounds in 50 gallons of water, both with and without casein spreader. The lead arsenate used was guaranteed to contain 31 per cent of As_2O_5 . The tests in 1923 were incomplete, owing to a scarcity of larvae, and only the results from two strengths of lead arsenate are given in Table 3. A complete series of tests was made in 1924, and the results are given in Table 4.

In 1923 the present writer made a number of laboratory tests with the codling moth, using a prepared casein spreader containing approximately 20 per cent of casein and 80 per cent of hydrated lime. This was added to the standard dilution of powdered lead arsenate at the rate of one-fourth pound and one-half pound to 50 gallons. A test was also made with three applications of the latter strength sprayed on within a few minutes of each other. It was thought that a thicker coat of spray might be obtained in this way. These tests are reported in Table 1, and show a decided improvement in control where the spreader was added. In test 2, with one-fourth pound of spreader, only a few more than half as many larvae entered the fruit as with the standard treatment in test 1, and the number of stings was slightly less. In test 3, with double the amount of spreader, the results were not as good as in test 2, but were better than in test 1. Test 4, sprayed three times, gave approximately the same results as test 3.

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1	Lead arsenate, 1-50; no spreader.....	3	40	26.7	34	22.7	49.4	0.85
2	Lead arsenate, 1-50; casein spreader, $\frac{1}{8}$ -50.....	3	22	14.7	30	20.0	34.7	1.36
3	Lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	28	18.7	26	17.3	36.0	.93
4	Lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50; sprayed 3 times.....	3	20	13.3	20	13.3	26.6	1.00
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The present writer made a number of tests with the codling moth, using powdered lead arsenate at various strengths from one-half pound to 3 pounds in 50 gallons of water, both with and without casein spreader. The lead arsenate used was guaranteed to contain 31 per cent of As_2O_5 . The tests in 1923 were incomplete, owing to a scarcity of larvae, and only the results from two strengths of lead arsenate are given in Table 3. A complete series of tests was made in 1924, and the results are given in Table 4.

In these tests the number of wormholes was reduced, and the number of stings generally increased as the strength of the lead arsenate was increased. The total number of blemishes was not very greatly reduced by the increase in the amount of poison used. The results obtained with powdered lead arsenate used at a strength of 1-50 and 2-50 are of particular interest, since there is a tendency at the present time to double the amount of material used. Both in 1923 and 1924 (Tables 3 and 4), increasing the amount of lead arsenate from 1-50 to 2-50 reduced the number of wormholes or the total number of blemishes very little. It is possible that the efficiency of the double-strength spray is reduced less rapidly than that of the single-strength as time goes on after it is applied, but this is a point that can not be tested with this type of experimentation. It is not safe to draw conclusions from these tests as to the optimum amount of lead arsenate for orchard use. It should be noted that, with casein spreader added, the double-strength spray resulted in less than half as many wormholes as the single-strength spray (Table 4, tests 8 and 9). In this particular case the number of stings seems to be an exception to the general tendency for the stings to increase as the strength of the spray is increased.

TABLE 3.—Comparison of the effectiveness of different strengths of powdered lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1923

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, 1-50.....	4	38	19.0	47	23.5	42.5	1.24
2	Lead arsenate, 2-50.....	4	36	18.0	35	17.5	35.5	.97
3	Control (unsprayed).....	4	132	66.0	7	3.5	69.5	.05

TABLE 4.—Comparison of the effectiveness of different strengths of powdered lead arsenate when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1924

Test No.	Material	Number of tests	Number of wormholes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per wormhole
1	Lead arsenate, $\frac{1}{2}$ -50; no spreader.....	4	50	25.0	30	15.0	40.0	0.60
2	Lead arsenate, $\frac{1}{4}$ -50; no spreader.....	4	36	18.0	36	18.0	36.0	1.00
3	Lead arsenate, 1-50; no spreader.....	4	37	18.5	40	20.0	38.5	1.08
4	Lead arsenate, 2-50; no spreader.....	4	30	15.0	41	20.5	35.5	1.37
5	Lead arsenate, 3-50; no spreader.....	4	19	9.5	46	23.0	32.5	2.42
6	Lead arsenate, $\frac{1}{2}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	29	19.3	25	16.7	36.0	.86
7	Lead arsenate, $\frac{1}{4}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	17	11.3	34	22.7	34.0	2.00
8	Lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	9	6.0	37	24.7	30.7	4.11
9	Lead arsenate, 2-50; casein spreader, $\frac{1}{4}$ -50.....	3	4	2.7	28	18.7	21.4	7.00
10	Lead arsenate, 3-50; casein spreader, $\frac{1}{4}$ -50.....	3	3	2.0	42	28.0	30.0	14.00
11	Control (unsprayed).....	4	110	55.0	4	2.0	57.0	.04

LIGHT AND HEAVY APPLICATIONS OF LEAD ARSENATE

The general opinion has been that where no spreader is used, it is more desirable to cover the fruit evenly with fine drops of spray than to "overspray" it and leave coarse drops on the surface with apparently unprotected areas between them. In Table 5 the results of

tests of this nature are recorded. In tests 1 and 4 (light applications) the fruit was thoroughly covered with small drops of spray, whereas in tests 2 and 5 (heavy applications) it was very much oversprayed and much of the spray dripped off. Most of the spray left on the fruit collected in large drops. The results in 1923 and 1924 are rather in favor of the heavy application, although the difference is not great, and, curiously, the number of stings is reduced more than the number of worms by the heavy application. From the standpoint of the ratio of stings to wormholes, the light application was better than the heavy application.

TABLE 5.—Comparison of the effectiveness of light and heavy applications of powdered lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1923 and 1924.

Wash., 1923 and 1924.

1923

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate, 1-50; light application.....	4	37	18.5	54	27.0	45.5	1.46
2	Lead arsenate, 1-50; heavy application.....	4	36	18.0	36	18.0	36.0	1.00
3	Control (unsprayed).....	4	132	66.0	10	5.0	71.0	.08

1924

4	Lead arsenate, 1-50; light application.....	3	16	10.7	44	29.3	40.0	2.75
5	Lead arsenate, 1-50; heavy application.....	3	13	8.7	29	19.3	28.0	2.23
6	Control (unsprayed).....	3	71	47.3	11	7.3	54.6	.15

PASTE VERSUS POWDERED LEAD ARSENATE

The powdered lead arsenate has almost entirely displaced the paste form, but there has been some question as to whether or not it is as effective. Scott and Siegler (16) obtained practically identical results in laboratory tests against the fall webworm with paste and powdered lead arsenate used at equivalent strengths, and also in orchard tests against the codling moth. Siegler and Plank (18) used paste lead arsenate in 1915 and powdered lead arsenate in 1916, 1917, and 1918, in orchard tests against the codling moth. It is difficult to compare tests made in different years, but the results do not indicate any advantage of one form of lead arsenate over the other. De Sellem (4) obtained somewhat better results with powdered lead arsenate at 1 pound to 50 gallons than with paste lead arsenate at 2 pounds to 50 gallons.

The paste lead arsenate was tested by the writer in 1924. Since the manufacturer of the particular brand employed recommended its use at 7 pounds to 200 gallons instead of the customary 8 pounds, it was used at that strength and compared with the powdered form at an equivalent strength—that is, at seven-eighths pound to 50 gallons—and also at the usual 1 pound to 50 gallons. This experiment, recorded in Table 6, does not indicate that the paste form is superior to the powdered, although the results with the paste were slightly better than those with an equivalent amount of powder. The paste lead arsenate used in this test contained 15.21 per cent of As_2O_5 , and the powdered lead arsenate was guaranteed to contain 31 per cent of As_2O_5 .

TABLE 6.—Comparison of effectiveness of paste and powdered lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1924

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Powdered lead arsenate, 1-50; no spreader.....	5	48	19.2	57	22.8	42.0	1.19
2	Powdered lead arsenate, $\frac{1}{8}$ -50; no spreader.....	5	47	18.8	74	29.6	48.4	1.57
3	Paste lead arsenate, $1\frac{3}{4}$ -50; no spreader.....	5	40	16.0	68	27.2	43.2	1.70
4	Powdered lead arsenate, 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	13	8.7	26	17.3	26.0	2.00
5	Powdered lead arsenate, $\frac{1}{8}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	20	13.3	40	26.7	40.0	2.00
6	Paste lead arsenate, $1\frac{3}{4}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	16	10.7	30	20.0	30.7	1.88
7	Control (unsprayed).....	5	140	56.0	14	5.6	61.6	.10

LEAD ARSENATE IN COMBINATION WITH LIME-SULPHUR AND OIL EMULSIONS

It is often desired to combine the summer strength lime-sulphur used for mildew and other fungous diseases with the lead arsenate, and the increasing use of summer oil sprays suggested the possibility of combining these with the lead arsenate. Accordingly, these combinations have been tested, acid lead arsenate being used.

According to Robinson (14), adding lime-sulphur to acid lead arsenate increases the amount of soluble arsenic and reduces the amount of arsenic oxide (As_2O_5). A large amount of lead sulphide (PbS) is also produced. Gray (6) questions the advisability of mixing lime-sulphur with acid lead arsenate. Sanders and Brittain (15) report that in field tests with various insect larvae the addition of lime-sulphur to the lead arsenate reduced its effectiveness, and Scott and Siegler (16) found that the killing action of the arsenical was slower when lime-sulphur was added than when the arsenical was used alone. Cook and McIndoo (3) obtained somewhat better results when the lead arsenate was used alone than when lime-sulphur was used with it. The writer, in various orchard tests against the codling moth at Yakima and Wenatchee, Wash., the results of which have not been published, has obtained poorer results when lime-sulphur was added than when the lead arsenate was used alone.

In the laboratory tests reported in Tables 7 and 8, a very marked increase in the number of wormholes resulted from the addition of lime-sulphur, the number being nearly twice as great in 1923, and more than twice as great in 1924. The number of stings was correspondingly decreased, and there was little difference in the total number of blemishes.

The addition of lime to this combination spray has been shown by Robinson (14) to prevent any reaction between the lime-sulphur and the lead arsenate, and Regan (13, p. 47) reports that the addition of casein spreader (a mixture of casein and lime) prevents this reaction. In test 3 (Tables 7 and 8) the casein spreader was used, it being placed in the water before the other ingredients were added. No reaction was noticeable, and the results are much better than those in test 2 where the combination spray was used without spreader, and even better than in test 1 where lead arsenate was used alone. This latter difference may be due entirely to the better coating obtained with the spreader.

In 1923, a lubricating-oil-emulsion made with soap according to W. W. Yothers's formula (23, p. 19) was mixed with the lead arsenate. Four tests were made, with rather variable results, the average, as shown in Table 7, test 4, being somewhat poorer than with the lead arsenate alone. Gray (6) states that soap and lead arsenate are incompatible, and Pinckney (12) found that soap increased the amount of soluble arsenic, but he does not mention whether it affects the toxicity of the arsenical. The variation in the results may have been due to a reaction between the lead arsenate and the soap, and, if so, it may be inadvisable to mix these two materials.

In 1924, the casein lubricating-oil emulsion prepared according to formula No. 3 of Burroughs and Grube (2) was used with lead arsenate in test 4 (Table 8). The results were superior to those obtained from the lead arsenate alone.

TABLE 7.—Comparison of the effectiveness of powdered lead arsenate alone and in combination with lime-sulphur and lubricating-oil emulsion, when used against the larva of the codling moth, Yakima, Wash., 1923

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate, 1-50; no spreader.....	4	43	21.5	46	23.0	44.5	1.07
2	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50.....	4	74	37.0	23	11.5	48.5	.31
3	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50; casein spreader, ¼-50.....	4	47	23.5	30	15.0	38.5	.64
4	Lead arsenate, 1-50; soap-oil emulsion, ½-50.....	4	55	27.5	24	12.0	39.5	.44
5	Control (unsprayed).....	4	138	69.0	8	4.0	73.0	.06

TABLE 8.—Comparison of the effectiveness of powdered lead arsenate alone and in combination with lime-sulphur and lubricating-oil emulsion, when used against the larva of the codling moth, Yakima, Wash., 1924

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate, 1-50; no spreader.....	4	31	15.5	54	27.0	42.5	1.74
2	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50.....	3	50	33.3	23	15.3	48.6	.46
3	Lead arsenate, 1-50; commercial 30° lime-sulphur, 1-50; casein spreader, ¼-50.....	3	12	8.0	23	15.3	23.3	1.92
4	Lead arsenate, 1-50; casein-oil emulsion, ½-50.....	4	21	10.5	43	21.5	32.0	2.05
5	Control (unsprayed).....	4	84	42.0	16	8.0	50.0	.19

VARIOUS ARSENICALS

Arsenicals other than lead arsenate have been used extensively in laboratory and field tests, chief among them being calcium arsenate and zinc arsenite. Paris green was formerly used, but owing to its poor adhesive qualities and its tendency to burn the foliage, it is now seldom used on fruit trees.

A review of some of the reports of tests of calcium arsenate shows a considerable diversity of results. Wilson (22) noted no difference in

the effect of calcium arsenate and lead arsenate on tent caterpillars. Lovett and Robinson (7) obtained equal results with the two materials used against leaf-feeding insects, as did Scott and Siegler (16), but the latter found that the calcium arsenate killed somewhat more slowly. Scott and Siegler's results with calcium arsenate in orchard tests against the codling moth indicated it to be inferior to the lead arsenate in two tests and about equal to it in a third test. W. M. Scott (17) reported that both the paste and the powdered calcium arsenate were equal to lead arsenate in orchard tests, and De Sellem (4) found the same thing to be true under favorable conditions. Lovett (8) obtained better results with calcium arsenate than with lead arsenate in the orchard, but Siegler and Plank (18) and Newcomer (11) obtained inferior results under wormy conditions. Regan (13, p. 48), testing calcium arsenate against the fruit-tree leaf roller, obtained poor results as compared with those obtained with lead arsenate.

Cook and McIndoo (3) made extensive experiments with various arsenicals against leaf-feeding insects. They obtained results comparable to those with lead arsenate with only one of six samples of commercial calcium arsenate tested. Paris green gave uniformly better results than the lead arsenate, and zinc arsenite gave somewhat poorer results. Melander (9, p. 23-24), reported very good results with zinc arsenite, under favorable conditions, but they were not equal to those obtained with lead arsenate.

Tables 9 and 10 give the results obtained by the writer with various arsenicals at Yakima, Wash., in 1923 and 1924. The powdered zinc arsenite, which was guaranteed to contain 30.5 per cent of arsenic expressed as metallic arsenic, was decidedly inferior to the lead arsenate when used at 1 pound to 50 gallons, and was still inferior when used at 2 pounds to 50 gallons.

Paris green, guaranteed to contain more than 50 per cent arsenious trioxide, was used in 1923, at one-half pound to 50 gallons, with an equal amount of lime, and also with casein spreader, and it produced slightly better results than lead arsenate. In 1924 it was used at two-fifths pound to 50 gallons, with an equal amount of lime, and also with casein spreader, and the results were not quite as good as with lead arsenate.

Powdered calcium arsenate, guaranteed to contain not less than 40 per cent arsenic pentoxide, was used in 1923 at three-fourths pound to 50 gallons, a strength equivalent to 1 pound of powdered lead arsenate to 50 gallons. The results with the calcium arsenate were decidedly inferior to those with lead arsenate, more than twice as many larvae entering the fruit where no spreader was used, and nearly three times as many where the casein spreader was added. In 1924, a paste calcium arsenate was tested, which analyzed 15.19 per cent arsenic oxide. This was used at 2 pounds to 50 gallons, and the results were practically identical with those obtained from the use of powdered lead arsenate at 1 pound to 50 gallons. When casein spreader was added, however, the results with calcium arsenate compared with lead arsenate were not nearly so good, indicating a possible reaction between the lime in the casein spreader and the calcium arsenate.

TABLE 9.—*Comparison of the effectiveness of various arsenicals when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1923*

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate (powdered), 1-50.....	4	47	23.5	47	23.5	47.0	1.00
2	Zinc arsenite (powdered), 1-50.....	4	83	41.5	39	19.5	61.0	.47
3	Paris green, $\frac{1}{2}$ -50; lime, $\frac{1}{2}$ -50.....	3	27	18.0	29	19.3	37.3	1.07
4	Calcium arsenate (powdered), $\frac{3}{4}$ -50.....	4	97	48.5	30	15.0	63.5	.31
5	Lead arsenate (powdered), 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	15	10.0	33	22.0	32.0	2.20
6	Zinc arsenite (powdered), 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	39	26.0	32	21.3	47.3	.82
7	Paris green, $\frac{1}{2}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	13	8.7	29	19.3	28.0	2.23
8	Calcium arsenate (powdered), $\frac{3}{4}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	44	29.3	44	29.3	58.6	1.00
9	Control (unsprayed).....	4	128	64.0	10	5.0	69.0	.08

TABLE 10.—*Comparison of the effectiveness of various arsenicals when used with and without casein spreader against the larva of the codling moth, Yakima, Wash., 1924*

Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate (powdered), 1-50.....	3	35	23.3	42	28.0	51.3	1.20
2	Zinc arsenite (powdered), 1-50.....	3	59	39.3	14	9.3	48.6	.24
3	Zinc arsenite (powdered), 2-50.....	3	42	28.0	23	18.7	46.7	.67
4	Paris green, $\frac{3}{4}$ -50; lime, $\frac{3}{4}$ -50.....	3	39	26.0	33	22.0	48.0	.85
5	Calcium arsenate (paste), 2-50.....	3	35	23.3	43	28.7	52.0	1.23
6	Lead arsenate (powdered), 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	11	7.3	47	31.3	38.3	4.27
7	Zinc arsenite (powdered), 1-50; casein spreader, $\frac{1}{4}$ -50.....	3	28	18.7	32	21.3	40.0	1.14
8	Zinc arsenite (powdered), 2-50; casein spreader, $\frac{1}{4}$ -50.....	3	16	10.7	37	24.7	35.4	2.31
9	Paris green, $\frac{3}{4}$ -50; casein spreader, $\frac{1}{4}$ -50.....	3	18	12.0	35	23.3	35.3	1.94
10	Calcium arsenate (paste), 2-50; casein spreader, $\frac{1}{4}$ -50.....	3	30	20.0	22	14.7	34.7	.73
11	Control (unsprayed).....	3	94	56.0	8	5.3	61.3	.10

CALCIUM ARSENATE

As already indicated, the results obtained by various investigators with calcium arsenate have been rather variable. Apparently this material may be depended on to give good control where the results are secured within a few days. This is particularly true of leaf-feeding insects, which eat comparatively large quantities of food, and which may not ingest a lethal dose of poison for several days. In the case of the codling moth, where the poison is expected to be effective over a period ranging from 10 days to a month, and where the insect does only a limited amount of feeding on the poisoned surface and must therefore take in a lethal dose within a short time, the control has been quite variable. This has evidently been due partially to a varying degree of infestation, but the work of Cook and McIndoo (3) would indicate also a varying degree of effectiveness in different brands of calcium arsenate. In order to test this point with the codling moth, four brands of powdered calcium arsenate

were used in 1923, and one brand of paste calcium arsenate in 1924. Comparisons were made with powdered lead arsenate used at an equivalent strength.

The powdered calcium arsenates used in 1923 contained the following percentages of arsenic pentoxide: Brand A, not less than 40 per cent by guarantee; brand B, 39.93 per cent, by analysis; brand C, 39.21 per cent, by analysis; and brand D, not less than 40 per cent, by guarantee. Table 11 shows that all of these samples were inferior to the lead arsenate in controlling the codling moth. Brands B and D were better than the other two, and these were fluffier and apparently more finely ground than brands A and C.

In 1924, a paste calcium arsenate was tested (brand E) which contained, by analysis, 15.19 per cent of arsenic pentoxide. The results obtained with this brand at 2 pounds to 50 gallons were almost identical with those obtained from an equivalent amount of powdered lead arsenate. Used at 1 pound to 50 gallons, the control obtained was somewhat poorer, but still fair. The addition of casein spreader to this calcium arsenate evidently interfered with its action, as the results were much inferior to those with lead arsenate and casein spreader. These results are recorded in Table 11.

Apparently there is a considerable difference in the effectiveness of different brands of calcium arsenate, and this difference is not due to a variable amount of arsenic present. It is possibly a physical difference, some kinds adhering better than others. The results with brand E indicate that a calcium arsenate can be made equal to lead arsenate in controlling the codling moth.

TABLE 11.—Comparison of the effectiveness of various brands of calcium arsenate with lead arsenate when used against the larva of the codling moth, Yakima, Wash., 1923 and 1924

1923								
Test No.	Material	Number of tests	Number of worm-holes	Per cent wormy	Number of stings	Per cent stung	Per cent total blemishes	Number of stings per worm-hole
1	Lead arsenate (powdered), 1-50.....	3	21	14.0	47	31.3	45.3	2.24
2	Calcium arsenate (brand A), $\frac{3}{4}$ -50.....	3	51	34.0	46	30.7	64.7	.90
3	Calcium arsenate (brand B), $\frac{3}{4}$ -50.....	3	45	30.0	43	28.7	58.7	.96
4	Calcium arsenate (brand C), $\frac{3}{4}$ -50.....	2	42	42.0	25	25.0	67.0	.60
5	Calcium arsenate (brand D), $\frac{3}{4}$ -50.....	2	29	29.0	38	38.0	67.0	1.31
6	Control (unsprayed).....	3	119	79.3	5	3.3	82.6	.04
1924								
7	Lead arsenate (powdered), 1-50.....	3	35	23.3	42	28.0	51.3	1.20
8	Calcium arsenate (brand E), 1-50.....	3	46	30.7	33	22.0	52.7	.72
9	Calcium arsenate (brand E), 2-50.....	3	35	23.3	43	28.7	52.0	1.23
10	Lead arsenate (powdered), 1-50; casein spreader, $\frac{3}{4}$ -50.....	3	11	7.3	47	31.3	38.6	4.27
11	Calcium arsenate (brand E), 1-50; casein spreader, $\frac{3}{4}$ -50.....	3	67	44.7	21	14.0	58.7	.31
12	Calcium arsenate (brand E), 2-50; casein spreader, $\frac{3}{4}$ -50.....	3	30	20.0	22	14.7	34.7	.73
13	Control (unsprayed).....	3	84	56.0	8	5.3	61.3	.10

SUMMARY

The difficulty of obtaining uniform working conditions when making orchard tests for the control of the codling moth has suggested the desirability of more exact laboratory methods. The method of spraying individual apples and placing a given number of larvae on them has been employed in the investigation reported in this paper. It is not possible to investigate all phases of codling-moth control by this method, but it is believed that certain comparisons may be made more accurately in this way than by field tests. It is to be understood that the tests here reported show the effect of the various sprays only when freshly applied.

Almost without exception, the addition of casein spreader to lead arsenate or to other arsenicals or combination sprays materially improved the control obtained. The smallest amount of casein spreader used, one-eighth of a pound to 50 gallons, gave better control when used with lead arsenate than any larger amount tested.

Increasing the strength of the lead arsenate reduced the number of wormholes and generally increased the number of stings, the total number of blemishes being reduced very little.

A heavy application of lead arsenate gave somewhat better results than a light application.

No appreciable difference was observed in the effectiveness of equivalent amounts of paste and powdered lead arsenate.

The addition of lime-sulphur to acid lead arsenate materially reduced its efficiency, but this reduction was overcome by the use of casein spreader with the combination spray. The lime in the spreader apparently prevents or retards the usual reaction between lime-sulphur and acid lead arsenate.

The addition of a lubricating-oil emulsion containing soap as an emulsifier produced variable results, the average being poorer than those obtained with lead arsenate alone. A lubricating-oil emulsion containing casein as an emulsifier did not have this effect, the control being somewhat improved over that obtained from lead arsenate alone.

Zinc arsenite was not as effective as lead arsenate. Paris green, with lime or with casein spreader, gave slightly better results in 1923, but in 1924 the control did not quite equal that obtained from lead arsenate. Powdered calcium arsenate gave very poor control, although four brands were tested. A paste calcium arsenate equalled an equivalent amount of powdered lead arsenate.

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LIFE HISTORY OF THE HICKORY SPIRAL BORER, *AGRILUS ARCUATUS* SAY¹

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INTRODUCTION

In the eastern part of the United States there are several species of beetles the larvae and adults of which kill hickory and pecan branches and young trees by girdling or otherwise severing the wood in a vital place. In all cases it seems that the purpose of the insect in doing the injury is to provide itself with dead or dying wood in which to undergo part or all of its development. This paper deals with one of these beetles, which may be called the hickory spiral borer because of the peculiar winding burrow which is the chief injury that it does to trees. In many instances medium-sized branches of bearing trees are killed by this borer, which reduces the crop of nuts, but the principal loss is in the injury to small trees. Young hickory trees in forests, in orchards, and on lawns, and especially in nurseries where seedlings and grafted trees are produced for planting, are liable to attack.

This beetle belongs to the family Buprestidae, the members of which are often called flat-headed borers or metallic wood borers. The term "flat-headed" refers to the broad, flattened front segments of the grubs of some species; and the term "metallic" to the lustrous, metallic colors often displayed by the beetles. The larvae of the many species of *Agrilus*, to which genus the hickory spiral borer belongs, mine in the twigs, stems, roots, and beneath the bark of trunks and branches of numerous kinds of trees and smaller plants. The group includes such familiar pests as the raspberry cane borer (*Agrilus ruficollis* Fab.), the two-lined chestnut borer (*A. bilineatus* Web.), the bronze birch borer (*A. anxius* Gory), and the Pacific oak twig girdler (*A. angelicus* Horn).

IDENTITY AND DISTRIBUTION

According to W. S. Fisher of the Bureau of Entomology of the United States Department of Agriculture, the forms of *Agrilus* related to *Agrilus arcuatus* are closely allied but not sufficiently well characterized to identify them with certainty. The form which feeds on hickory and pecan is believed to represent only one species, and it is treated in this paper under the name *arcuatus*. Certain varieties of *arcuatus* have been recognized by Le Conte, but they resemble one another so closely that it seems likely that misidentifications have been made. For this reason it does not seem desirable to summarize the literature which has appeared under the name *arcuatus* or its varieties.

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This confusion of names makes it uncertain as to how widely the species here called the hickory spiral borer is distributed, and whether other trees than hickory and pecan are sometimes attacked. There

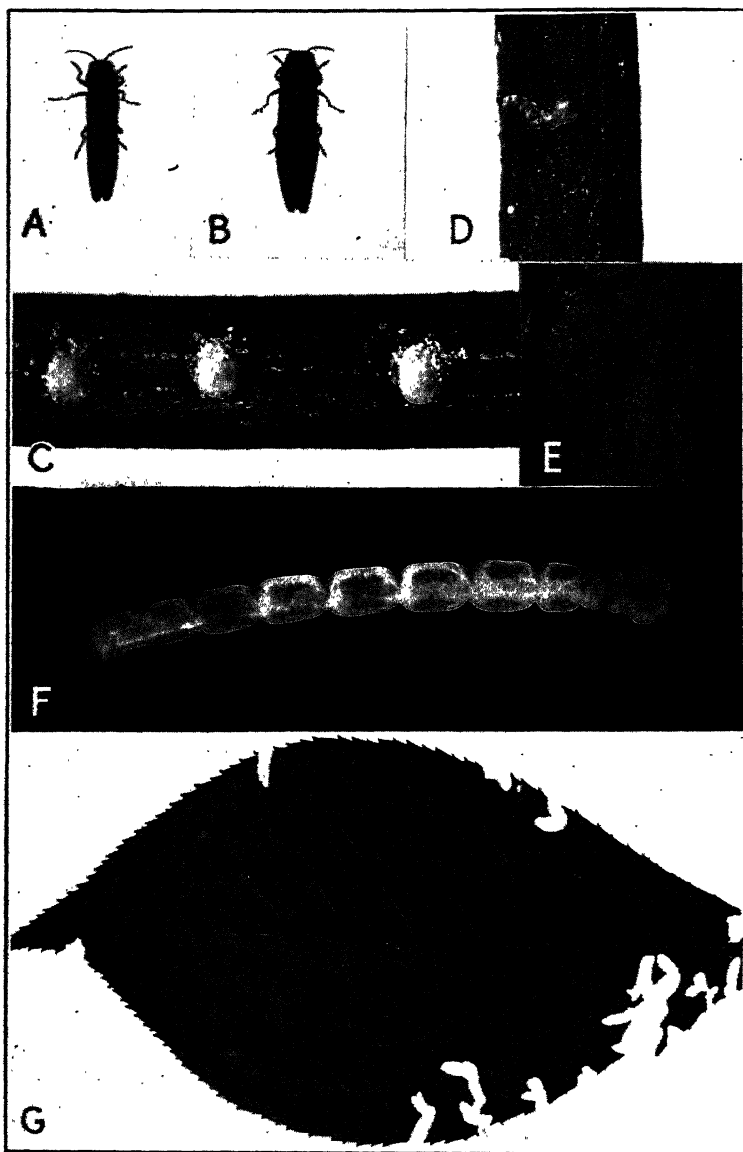


FIG. 1.—Hickory spiral borer: A, male beetle, $\times 2$; B, female beetle, $\times 2$; C, eggs, $\times 5$; D, larva issuing from egg, $\times 5$ (the eggshell has been removed to the left from over the larva in order to disclose the hatching larva); E, exit hole of beetle, $\times 2$; F, larva, $\times 4$; G, hickory leaf showing feeding marks of the beetle, natural size

seem to be valid records of its occurrence in New York, Pennsylvania, and Virginia. The present writer has collected the insect or has seen specimens of its work at Morgantown, Pickens, French Creek,

Buckhannon, Moorefield, Great Cacapon, and Clarksburg, W. Va.; and at Pulaski, Richmond, Petersburg, and Round Hill, Va. Severed hickory branches, apparently the work of this insect, were found by the writer in the vicinity of Storrs, Conn.

NATURE AND EXTENT OF INJURY

The hickory spiral borer hatches from an egg laid on the bark of a twig (fig. 1, C), and lives as a larva in the tree from 22 to 23 months, the period including two entire winters. The larva (fig. 1, F) spends most of the summer burrowing just under the bark, occasionally going deeper into the wood and sometimes following the pith for short distances. Its general course in the wood is downward. Each winter it makes a winding, concentric cut from the inner bark to the heart of the branch or stem, the coils of the thin burrow joining and completely severing the wood except for the bark and sometimes a slender fiber of wood at the heart (fig. 2, C and D). These spiral burrows are in process of making in late autumn, in warm periods in winter, and in the early spring. The first of the two spiral burrows which each insect makes severs a small twig or terminal, and the second severs a larger part of the wood. The portion above the spiral wound dies in the spring before the foliage appears, the injury becoming apparent as the rest of the tree puts forth leaves (fig. 3). Trees and branches from the diameter of a lead pencil to nearly 2 inches in diameter are severed.

The borer is native to the eastern forests where hickory trees abound, and in several instances it has been observed injuring hickory and pecan trees in nurseries and nut-tree orchards in the eastern part of the country. In a nursery at Petersburg, Va., in 1923 and 1924 it did serious damage to seedling and grafted hickories and pecans. In one small block of 5-year-old hickory seedlings in this nursery an hour's search revealed 54 trees with their main stalks recently severed by 2-year-old larvae, and an equal number of twigs and terminals similarly injured by 1-year-old larvae. The trees which the older larvae had severed were set back several years in growth, many of them being practically ruined. Oftentimes such young trees with trunks thus severed will put forth another shoot which will in turn be cut off within a few years. In many instances in localities where the insect is abundant, hickory bushes which have become stunted by frequent pruning will show from 5 to 10 fresh and old wounds. The greatest injury to plantations has been observed near woods in which hickory grows. However, trees in open fields, as well as in the shadow of forest, are liable to attack.

LIFE HISTORY

THE EGG

The eggs (fig. 1, C) are flat, disklike, somewhat irregular in shape and size, and are glued firmly to the smooth bark of twigs. They resemble quite closely the shield of a small scale insect. When first laid the egg is smooth and pale yellowish green, but before hatching it becomes slightly wrinkled and almost black. The diameter ranges from 0.8 mm. to 1.1 mm. When laid they are partially concealed with a light covering of grains of excrement (fig. 1, C), but the covering soon drops away, and as the egg deepens in color, a

transparent film appears around it. About a dozen eggs collected at different times hatched in 26 and 27 days. When confined in cages the beetles often laid several eggs in a more or less compact group, but in the field not more than one egg was found in a place.

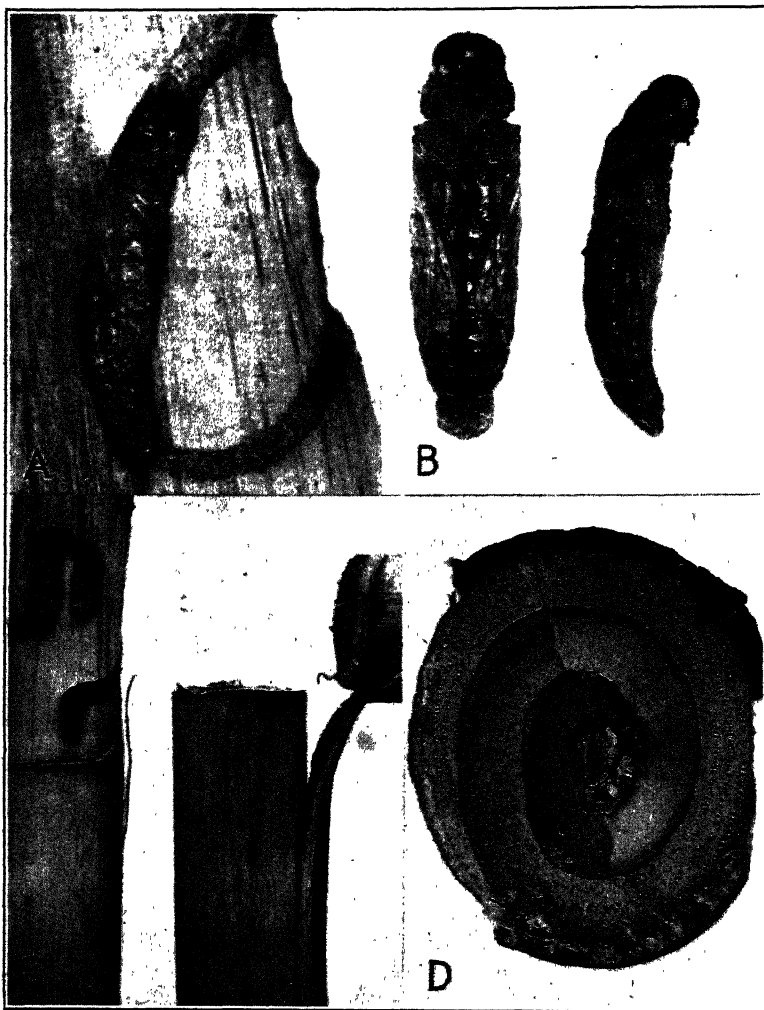


FIG. 2.—Hickory spiral borer: A, pupa in natural position, $\times 5$; B, pupae, $\times 5$; C, hickory stalks showing the burrows; D, hickory branch at point of severance showing spiral girdle of larva, $\times 2$

THE LARVA

The larva (fig. 1, F) is a slender, flat, legless grub, full-grown specimens being from 15 mm. to 20 mm. long and about 2 mm. wide. The color is yellowish white, like that of the wood in which it feeds, except that the small mouth parts and the tail forceps are dark brown or black. It is bare of hairs, save for a small patch of short, whitish bristles at the base of the tail forceps. It is sluggish in movement at all times.

The larva leaves the egg in midsummer, eating its way through the bottom of the egg and directly into the twig. In the twig it makes elongate, threadlike burrows next to the bark and through the wood. Late in autumn it begins a spiral burrow which even-



FIG. 3.—Top of a young hickory tree killed by larvae of the hickory spiral borer

tually severs the wood and kills the terminal above. The spiral burrow may be completed in the autumn, or in the following spring. In either case the larva spends the winter in and about the spiral wound. In all its mining the borer packs the gallery behind with fine wood dust.

With the coming of warm weather the borer works below the severed point, first moving downward for an inch or two in the pith and then tunneling out to the bark. Through the summer it proceeds downward a foot or more, feeding with its back to the inner bark and leaving a shallow but relatively wide burrow packed with wood-colored dust. Late in the season it changes its course abruptly and, with its side to the bark, cuts a thin, symmetrical ring around the branch (fig. 2, C and D). When the first circuit is completed it directs its boring operations spirally inward in the same plane, encircling the stem again, and it continues the spiral burrowing until the heart is reached, when the terminal above will break off with the application of slight force. When this second spiral boring is completed the borer turns upward and eats its way from the heart directly to the bark at a point about half an inch or less above the outer tunnel of the spiral boring (fig. 2, C). By the time it has arrived at the bark, spring has returned, and the larva is then nearly full-grown. It then proceeds to make a crooked burrow a few inches in length just beneath the bark (fig. 2, C) after which it forms a crescent-shaped pupal chamber, the ends of the chamber extending to the bark and the bottom curving toward the heart of the wood (fig. 2, A). Both ends of the chamber are filled with dust, and the larva occupies the somewhat enlarged space between. The larva soon shrinks to about half its former length, and in about a week it changes to the pupal stage.

THE PUPA

The pupa (fig. 2, A and B) when first formed is a delicate, white, somewhat curved object, the back being flattish and the underparts bearing outlines of the antennae, wings, and legs. It ranges from 9 to 12 mm. in length, and from 2 to 3 mm. in width. Within a week the eyes begin to darken, and soon thereafter the head, thorax, and other parts of the body change to purplish black, the shades of color deepening until the beetle stage is reached. The pupae are present in May and June, the pupal stage of development lasting about 20 days. The pupa rests in an almost vertical position in a pupal chamber which curves inward and downward from the bark, usually to the heart of the branch. The middle section of the chamber has been enlarged somewhat by the larva for the more comfortable accommodation of its body (fig. 2, A).

THE ADULT

The adults (fig. 1, A and B) are dark and slender, the males averaging considerably smaller than the females. The head and thorax of the male are greenish bronze, the elytra are purplish black, and the underparts are brassy; the female is bronze in color effect throughout. The average length of the male is about 8 mm., and that of the female about 10 mm. The beetles appear from May to July, are active only on warm days, and live for about two months. Most of the eggs are deposited in July and the first half of August.

The beetle makes its escape from the pupal chamber through a small D-shaped hole which it gnaws through the bark (fig. 1, E). This hole is usually from 1 to 3 inches above the spiral wound which kills the terminal. The beetles appear from May to July, emergence of individuals in a given locality occurring over a period of at least 25 days, and the season of emergence varying in different localities.

In the summer of 1924 a number of beetles were reared from hickory wood collected at Petersburg, Va., and French Creek, W. Va. Petersburg is approximately 115 miles south of French Creek, and is perhaps 1,400 feet nearer sea level than French Creek. As would be expected, the beetles from the more southerly locality, although belonging to the same generation, transformed and issued first. The beetles reared from the material collected at Petersburg numbered 19, and they issued from May 30 to June 13, the maximum emergence being from May 30 to June 10. The number reared from the material collected at French Creek was 111, and emergence continued from June 23 to July 17, the maximum emergence being from June 24 to June 30. The beetles from Petersburg appeared, on an average, from 20 to 30 days ahead of those from French Creek.

The males appear a few days before the females, and they also die first. In a lot of 10 pairs confined and fed in glass jars, all the females survived the males. Practically all the beetles left the wood in the morning and began to feed soon after they issued, making elongate notches and slits in the edges of the leaves (fig. 1, G). Mating took place within from 12 to 24 hours after emergence, and the first eggs were, as a rule, deposited from 10 days to two weeks later. A female in the act of egg laying was observed to first spend several minutes moving over the bark with the tip of her abdomen pressed against the surface as though searching for a suitable place to deposit her eggs. When such a place was found she devoted about a minute to scraping the spot with her anal tip to smooth or moisten the surface. She then laid an egg and immediately began to move the tip of her body rapidly across and in circles over the egg and the bark immediately surrounding it. This performance lasted 90 seconds, and when it was finished the egg was thinly covered with a transparent liquid, present in slight amount, which glued the egg to the bark.

On July 1, 1924, ten pairs of beetles which had recently issued in rearing jars and which had not begun to oviposit, were found in copulation, and they were confined by pairs in glass jars in an open insectary. They were provided daily with fresh hickory leaves for food and fresh hickory twigs on which to oviposit. A record was kept of the number of eggs laid by each female and the time of death of all individuals.

The 10 females laid 226 eggs, an average of 22.6 each. The greatest number laid by one female was 55, and the smallest number was 2. Eggs were laid during a period of 51 days. Two females of the lot issued from the wood on June 23, first mated on June 24, and lived until August 29, a period of 67 days. The greatest number of eggs laid in one day by the 10 females was 15; this was on July 27. The period of maximum egg production was from July 12 to August 8. The females, on an average, lived about three weeks longer than the males.

NATURAL ENEMIES

Several species of hymenopterous parasites were found killing the larvae of the borer, although a relatively small percentage of the insects were parasitized. These Hymenoptera included *Labena apicalis* Cress., determined by R. A. Cushman; and *Monogonogastra agrili* (Ashm.) and *Zatropus* sp. near *nigroaeneus* (Ashm.), determined by S. A. Rohwer.

METHODS OF CONTROL

It seems probable that the hickory spiral borer may be held in check in nurseries and other plantations of small hickory and pecan trees by dusting or spraying with arsenicals. Beetles kept in cages ate rather freely of the leaves with which they were provided, and they responded to the poison when it was given to them on sprayed foliage. In one instance 20 beetles, equally divided as to sex, were placed in a roomy cage over a live hickory branch that had been sprayed with lead arsenate at a strength of 2 pounds of powdered arsenate to 50 gallons of water. Two days later 9 of the beetles were dead, in 3 days 18 were dead, and the other 2 died in 4 and 5 days, respectively. In a near-by cage set up as a check 50 beetles were confined over an unsprayed branch, and not one of these died during the period of the test. Arsenicals for the control of the borer should be applied from the first to the last of June, according to the locality.

Infested young trees in nurseries and orchards should be pruned of the killed branches and terminals as soon as the leaves develop in the spring. By pruning thus early the insects will be collected with the prunings and may be destroyed by burning. Special care should be taken to cut off the small dead twigs that have been severed by the first-winter larvae. Such twigs should be clipped a few inches below the dead part in order to make sure of getting the borer, which starts to move down the stem at the beginning of warm weather. Twigs containing the 1-year-old larvae need not necessarily be burned, as the borers within them will die when the twigs are cut off. By so disposing of the young borers the more serious damage which they would inflict later may be avoided.

THE PINE BUTTERFLY, NEOPHASIA MENAPIA FELDER¹

By JAMES C. EVENDEN

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INTRODUCTION

Thousands of acres of yellow pine along the Little Salmon and Payette Rivers, Idaho, were severely defoliated by the pine butterfly (*Neophasia menapia* Felder) in 1922 and 1923. Much has been published concerning this insect, and very accurate descriptions of the various stages of its development have been given (7, 2, 4),³ but no data are available relative to its seasonal history, and conflicting ideas have been advanced as to the number of generations per season (1, 6). Therefore it seems desirable to publish the results of the last two years' field and laboratory study of the insect in Idaho, including brief descriptions of the egg, larva, and imago, together with its distribution and its hosts.

DISTRIBUTION AND HOSTS

Epidemics of the pine butterfly have been recorded from various sections of the Northwest (5, 7). The insect has also been collected in California, and it is found as far east as the eastern or front range of the Rocky Mountains. Briefly, it may be considered as present in all of the pine forests in the western part of the United States and Canada.

Practically all species of pine throughout the range of the insect are attacked to a greater or less extent, but western yellow pine (*Pinus ponderosa* Laws.) is the preferred host (fig. 1).

Fletcher (3) records the pine butterfly as severely injuring Douglas fir (*Pseudotsuga taxifolia* (Poir) Britt.) in the coast regions of British Columbia. The writer has no record of injury to trees other than to pine in Idaho.

DESCRIPTION

ADULT

Stretch (7) has very accurately described both the male and female. Nevertheless a brief description intended for field determinations may not be out of place in this paper. The pine butterfly very closely resembles the common cabbage Pieris. It has a wing expanse of about 43 mm.; antennae black; head and body black above and white beneath, covered with hairs (fig. 2, E).

MALE.—The scales of the fore wings of the male are pure white, except for the black markings on the tips and a streak along the costal vein. On the under side the same general markings occur.

The hind wings are white, except along the tips, which are lightly touched with black. On the under side the markings are heavier, and the black veins show through on the upper side.

¹ Received for publication Jan. 26, 1926; issued August, 1926.

² The writer wishes to acknowledge the assistance given by H. J. Rust, senior scientific aid, Bureau of Entomology, in conducting the rearing experiments in connection with the laboratory studies.

³ Reference is made by number (italic) to "Literature cited," p. 344.

FEMALE.—The scales of the fore wings of the female are of a light yellowish color. The black markings are like those of the male, with a black apical margin in addition. On the under side the markings are the same.

The hind wings have the same yellowish tint as the fore wings, but they differ from those of the male in having heavier black lines along the tips. More black appears on the under side, all of the veins being marked with broad lines. On many specimens, but not all, there are bright orange-red spots along the apical margin.

EGG

The eggs (fig. 2, A), $1\frac{1}{2}$ mm. in length, are laid along the pine needle, in rows of 5 to 20, at an angle of 45° pointing toward the

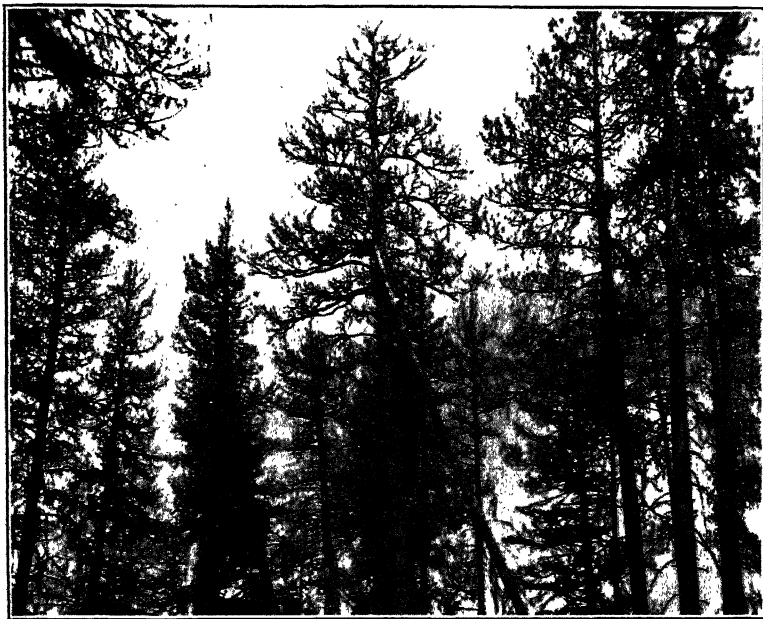


FIG. 3.—Stand of western yellow pine defoliated by the pine butterfly

end of the needle, and are firmly cemented together. Fletcher (3) has described them as the "most beautiful objects, resembling minute emerald-green Florence flasks, vertically lined with delicate lines and with a beaded rim of porcelain-white knobs."

LARVA

Although the larval stages have been described fully by Edwards (2), a brief description of the young and mature larvae is included here for the convenience of the reader in determining the insect in the field. The larva is 2 mm. in length as it hatches from the egg; body a pale green, and head shiny black. The mature larva (fig. 2, B) is approximately 25 mm. in length; body a dark green, with two white lateral stripes down each side; head pale green, dotted with raised white tubercles, each giving rise to a short hair; anal

shield similarly covered with white tubercles, produced behind into two blunt, well-separated projections; body skin covered with a fine pubescence; prolegs on abdominal segments 3, 4, 5, and 6 and the normal anal prolegs; crochets of prolegs biordinal, arranged in longitudinal band.

BIOLOGY AND HABITS

There is but one generation of the pine butterfly yearly in Idaho. This fact, however, is rather confusing to the casual observer or collector, since there is a marked overlapping of the seasonal history events by individuals of this one brood. Elevation and exposure have a marked influence on the development of the insect. Imagoes, especially males, are often seen at high elevations as late as October.

The overwintering eggs hatch about the time that the new needles begin to appear on the western yellow pine. This occurs during the first half of June, depending upon the season. The development of the young larvae is very slow during the first two weeks. They feed in clusters, encircling the needle, with their heads pointing toward the tip of the needle, making a tiny ring of black beads.

Only the fleshy part of the needle is eaten by the young larvae (fig. 3); but after the first molt the entire leaf is destroyed. During the first molt the shiny black head covering is shed, the subsequent color being a yellowish green. When the larvae are about half grown the habit of feeding in clusters is no longer continued, but often two or more may be found upon the same needle, especially if there is a shortage of food material. After the second molt growth is very rapid, and the larvae are approximately full-grown by the last of July, or about 50 days after hatching.

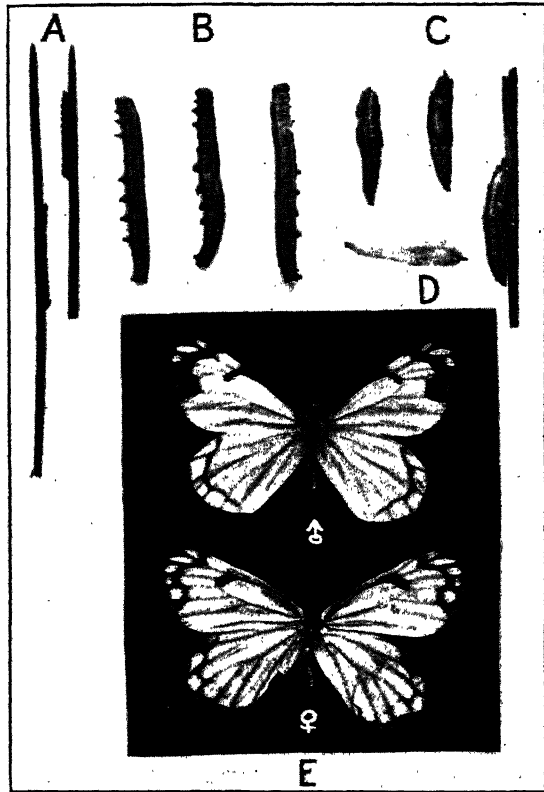


FIG. 2.—Pine butterfly: A, eggs; B, mature larvae; C, pupae; D, empty pupa case; E, imagoes

When mature, the larvae lower themselves by a silken thread, which sometimes is 75 feet or more in length, from the trees to the ground, where they pupate as chrysalids on shrubs, grasses, limbs, fences, tree trunks, or other objects. The pupal stage lasts from 15 to 20 days. Mating occurs almost immediately after the emergence of the adult, and oviposition takes place a few hours later. These eggs overwinter and hatch the following June.

The normal habit of the female is to deposit her eggs on the needles at the top of mature trees. However, in areas which have been se-



FIG. 3.—Larvae of the pine butterfly feeding on the foliage of western yellow pine

verely defoliated she apparently is forced to seek suitable foliage on younger trees or else migrate to other areas, which she does in numerous instances.

NATURAL ENEMIES

Although the area defoliated by the pine butterfly in 1923 was at least 25 per cent larger than that in 1922, when the outbreak first became serious, there was a heavy mortality caused by the natural enemies of the insect, and it was believed that the epidemic was at

an end. This belief was fully substantiated during the 1924 season, as it was practically impossible to find a larva, and no damage was observed that season.

The most important of the enemies of the pine butterfly was a parasitic ichneumonid (*Theronia fulvescens* Cress.) (fig. 4). This insect lays its eggs on the caterpillars, which, though severely weakened, are for the most part able to reach the pupal stage. The adult (fig. 4, C) emerges in September. The parasitized pupae can easily be distinguished from the others by their dark brown color, the normal pupae ranging from a pale to a dark green. A predacious hemipteran (*Podisus placidus* Uhler) was also present in large numbers, but the importance of this insect in the reduction of the present epidemic is not known. It is possible that these parasites are responsible for the cessation of the many sporadic outbreaks of the pine butterfly which have occurred in the past.

ECONOMIC IMPORTANCE

The economic importance of pine-butterfly epidemics is rather uncertain, as the degree of damage depends entirely upon the severity of the defoliation. Though the terminal buds were not injured by the in-

sects and new needles were produced after the two years' defoliation, a large percentage of the overmature, decadent trees were unable to recover from the injury received and they began to die in 1924 and 1925. There is no doubt but that the effects of the damage which occurred during this outbreak will continue to appear for several years, with a loss ultimately amounting to several hundred thousand dollars.

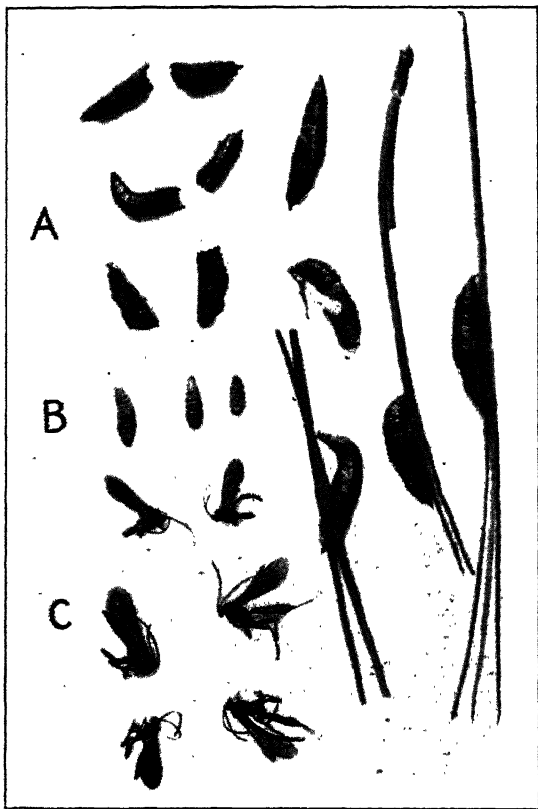


FIG. 4.—*Theronia fulvescens*, a parasite of the pine butterfly: A, parasitized pupal cases, some of which show emergence hole of parasite; B, larvae; C, imagoes

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SUSCEPTIBILITY OF THE BEAN TO THE VIRUS OF SUGAR-BEET CURLY-TOP¹

By EUBANKS CARNSNER

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The disease of beans (*Phaseolus vulgaris*) of a mosaic type, which is caused by the virus of curly-top in sugar beets, has not been described so far as the writer is aware. The only reference that he knows of in this connection is one by himself,² in which the bean was reported as one of the species which had been tested and found nonsusceptible. This statement was made on the basis of a test with the Pink bean, a popular California variety. Interest in the matter was revived when circumstantial evidence suggested the possibility that a disastrous epidemic disease of beans which occurred in Twin Falls County, Idaho, in 1924 might have resulted from infestation of the beans by the leaf hopper *Eutettix tenella* Baker, which transmits the virus of curly-top.

When the question as to the cause of the bean disease in Idaho was raised, seed samples of seven of the varieties of beans commonly grown in Twin Falls County were obtained for the purpose of making test inoculations. Greenhouse plantings were made at once of two of these varieties—the Montana White, or Great Northern, and Prolific Black Wax—and later the plants were inoculated. Viruliferous leaf hoppers were caged on several plants of each variety, and nonviruliferous leaf hoppers were caged on similar plants as controls. The plants on which viruliferous insects were caged became diseased, while the controls remained healthy. Later, to prove that the diseased beans had curly-top, nonviruliferous leaf hoppers were caged on them and then transferred to healthy beets. The beets to which these insects were transferred developed symptoms of curly-top.

The symptoms of the disease on the beans were marked dwarfing and distortion of the leaves which grew out after infection had occurred. These affected leaves became darker green than were the corresponding leaves of the check plants, and became puckered, with their edges curved downward (fig. 1). The puckering was probably due, as is often the case in curly-top beets, to the severer checking of the growth of the fibrovascular bundles than of the intervening mesophyll. The leaf veins showed slight clearing when viewed by transmitted light, as is shown conspicuously by affected beets, but no irregular swellings such as are so characteristic of the disease in beets were found.

The greenhouse inoculations indicated a difference in susceptibility to the disease between the two varieties, the Montana White seeming more resistant. To test this matter further, field plantings

¹ Received for publication Jan. 4, 1926; issued August, 1926.

² CARNSNER, E. SUSCEPTIBILITY OF VARIOUS PLANTS TO CURLY-TOP OF SUGAR BEET. Phytopathology 9: 413-421, illus. 1919.

were made of the two varieties mentioned, and also of five other varieties. Fifty to 75 plants of each variety were inoculated by caging four viruliferous leaf hoppers on each plant. One month after inoculation the following observations were recorded:



FIG. 1.—Curly-top of garden bean (*Phaseolus vulgaris*, var. Prolific Black Wax). Five viruliferous leaf hoppers were caged on the plant on the right on December 24, 1924. Five nonviruliferous leaf hoppers were caged on the plant on the left on the same day. (Photographed January 13, 1925)

The Montana White, or Great Northern, variety seemed rather resistant; the inoculated plants were only slightly smaller and less healthy in appearance than the uninoculated controls.

Prolific Black Wax was less resistant than Montana White, but was not as susceptible as the other five varieties.

Pencil Pod Black Wax was slightly more susceptible than Black Prolific.

Giant Stringless, Full Measure, and Improved Golden Wax seemed about equally susceptible and slightly more so than Pencil Pod Black Wax.

Black Valentine seemed most susceptible of all.

When the last observation was made on these field plants on September 17, about two-thirds of the plants in the row of Montana White were alive, and one plant was alive in the row of Prolific Black Wax, while in the rows of the five other varieties all plants were dead.

It is of interest to note in connection with these observations on resistance and susceptibility that, according to E. P. Brossard, county agent of Twin Falls County, the Montana White, as well as the Pink variety, was injured in decidedly less degree by the epidemic disease in that district than were other varieties, and that Black Valentine was the most seriously affected of all.

The writer is still uncertain whether the epidemic disease of beans in southern Idaho was curly-top or not. The epidemic was called to the writer's attention in the fall of 1924, and he had no opportunity to see affected plants in the field. However, certain lines of circumstantial evidence suggested that the disease might have been curly-top. One phase of this evidence which seems worth mentioning is the fact that the leaf hoppers invaded the cultivated areas of southern Idaho in enormous numbers in 1924; and the fact, observed by Severin³ and by C. F. Stahl and the writer (unpublished), that when the leaf hoppers fly into cultivated areas in immense numbers they at first settle down on practically any green vegetation. Aside from this observation and the reported similarity in varietal resistance, it might be mentioned that the symptoms as described to the writer by Brossard and another observer corresponded to the curly-top symptoms seen when viruliferous leaf hoppers were caged on healthy bean plants. In the season of 1925 no serious damage to beans from disease was reported in southern Idaho. The writer has no direct evidence to support the view, but he is inclined to think that the non-occurrence of the epidemic disease in that district in 1925 may be correlated with the fact that the number of the beet leaf hoppers was very much less in 1925 than in 1924, when there was a great abundance of them.

In regard to the occurrence of curly-top on beans in California, the writer has made no observations in commercial fields in that State. He is, however, authorized to quote the following statement made to him by Henry H. P. Severin, of the California Agricultural Experiment Station, who conducted studies in 1925 when the beet leaf hopper was exceedingly abundant in California:

It was demonstrated that many varieties of field beans, as well as a large number of other crops, were affected with curly-top under natural conditions. In the lower Salinas Valley where Small White beans were grown in the vicinity of a beet field which had been plowed under owing to curly-top, the beet leaf hoppers flew into the bean fields, and an average of 63 per cent

³ The following is quoted from a manuscript by H. H. P. Severin now being prepared: "San Joaquin Valley—Flights in middle valley: When the immense swarms of beet leaf hoppers flew into the cultivated regions on Apr. 14, 1919, they were found during the next day generally distributed on green vegetation, but later they congregated on their most favorable host plants for the purpose of feeding and egg laying."

of the bean plants showed typical symptoms of the disease on August 19-20. Bean plants showing curly-top symptoms were transplanted from the field to flower pots in the greenhouse. Noninfective beet leaf hoppers were allowed to feed on these bean plants for a period of three days or longer, and were then transferred to healthy sugar beets. In the cases where the beets developed curly-top, plainly the curly-top virus had been transmitted from bean plants naturally infected under field conditions. Noninfective leaf hoppers which had been allowed to feed on apparently healthy bean plants which had been transplanted from the field failed to produce curly-top in beets. Such leaf hoppers after feeding on healthy beans grown from seed in the greenhouse also failed to transmit the virus.

The question whether or not the leaf hopper finds the bean a favorable host plant is significant from the standpoint of the amount of damage to the crop that may be expected from curly-top. That the bean is not a favorable food plant for the insect is indicated by the fact that in cage experiments all of the leaf hoppers died within 17 days after being caged on the beans. Principally on this account the writer is inclined to the opinion that it is only in seasons when the leaf hopper is relatively very abundant that serious damage to the bean crop from curly-top may be expected, rather than that the disease will be a continual menace as it is to sugar beets.

QUANTITATIVE INHERITANCE IN PHASEOLUS¹

BY KARL SAX

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INTRODUCTION

The characters which involve productivity in plants are usually dependent upon so many factors that a satisfactory study of the inheritance and yield can be made only by a study of linkage relations with simple qualitative factors. This method of analysis should also show the interrelation of many of the factors involved in productivity.

MATERIALS AND METHODS

The parents selected for this investigation were the Symonds White bean, and a small-eyed bean selected from a cross of Improved Yellow Eye \times Small White. They are both of local origin, and differ in many characters. The Symonds White bean, of unknown parentage, is very productive, comparatively early, and bears very large white seeds. The small-eyed bean is unproductive, bearing small, partially pigmented seeds. Although the Symonds is a bush variety the plants are very large and are as tall as those of the small-eyed variety, which is a runner type. Data on the parental varieties and the F_2 segregates are given in Table 1. The mean values of the various parental distributions were calculated from frequency distributions with smaller units in case of yield per plant and seed weight.

The F_1 was very productive; the plants were of the runner type, and the seeds were large, completely pigmented, and mottled. The F_1 and F_2 and the parental varieties were grown in an insect-proof cage to prevent cross-pollination. In the F_2 the date of appearance of the first blossom was recorded for each plant. The length and breadth of the largest leaf on each plant was measured in order to get an index of leaf type. Leaf type was obtained by dividing the length of the leaf by the width. The F_2 segregates were also described as runner or bush. All runner beans were trained on strings to permit maximum development. All beans on each plant were harvested, and the total yield in grams and average seed weight in centigrams were calculated later in the year.

EXPERIMENTAL DATA

The F_2 plants segregated in a ratio of 27 mottled: 9 self-colored: 12 eyed: 16 white, indicating that simple independent factors are involved for the characters pigmented versus white, completely pigmented versus eyed, and mottled versus self-colored. The segregation of vine type is also probably dependent on a single-factor difference which is not linked with the seed-coat characters described, although the proportion of bush-type plants is considerably too large.

¹ Received for publication Jan. 27, 1926; issued August, 1926. This is Paper No. 173 from the Biological Laboratory of the Maine Agricultural Experiment Station. Approved for publication by the director of the station.

In studying the association of size characters with qualitative characters it is impossible to distinguish between a single factor which effects both qualitative and quantitative characters and two separate factors which are closely linked. If two factors are involved, their linkage relations can be approximately determined by growing the F_2 families, as Sirks² has done in his study of the inheritance of seed weight in the garden bean. So far as the study of the inheritance of size and yield characters are concerned, however, it makes little difference whether a single factor effects both qualitative and quantitative characters or two linked factors are involved. The relative differences between each homozygous class of F_2 segregates and the heterozygous class will also be the same regardless of the amount of crossing over between the two factors. In the following discussion we have assumed that the size factors are linked with the qualitative factors when the respective characters are associated.

If these quantitative characters are dependent upon a number of genetic factors for their expression, one would expect to find some linkage with one or more of the four independent characters involving seed-coat pattern or vine type. The data showing the relation between the qualitative and quantitative characters are shown in Table 2. In previous investigations it was found that seed weight was linked with mottling and pigmentation of the seed coat.³ In this cross there seems to be no linkage between seed weight and seed-coat pattern, although the difference between the parental varieties is very great.

TABLE 2.—Data showing segregation and relation of qualitative and quantitative characters in F_2 of *Phaseolus*

CLASSES OF SEGREGATES

Segregation in F_2	Mottled	Self	Eyed	White	Total number of plants	
Numbers obtained.....	142	46	80	79	347	P=.12
Theoretical for 27: 9:12:16.....	145	49	65	86	-----	-----
Vine type:						
Runner.....	89	35	56	56	236	-----
Bush.....	53	11	24	23	111	-----
					Runner	Bush
Seed weight.....	46.63±0.40	46.11±0.62	45.70±0.47	46.25±0.47	47.09±0.28	44.50±0.43
Yield.....	21.46±1.00	21.41±1.35	17.44±1.01	18.67±.90	24.21±.67	10.70±.61
Date of bloom.....	18.32±.16	19.96±.36	19.88±.25	18.70±.16	19.25±.13	18.42±.42
Leaf type.....	1.452±.008	1.502±.012	1.486±.013	1.466±.011	1.480±.006	1.452±.016

Yield per plant has also been found to be linked to some extent with seed-coat characters.⁴ In this cross the mottled and self-colored segregates are significantly more productive than the eyed and white segregates. This suggests that one or several of the factors for productivity are linked with the factor for extension of the pigment. If this is the case, then the white segregates should be more or less intermediate in productivity as compared with the mottled or self

² SIRKS, M. J. THE INHERITANCE OF SEEDWEIGHT IN THE GARDEN BEAN (*PHASEOLUS VULGARIS*).

1. *Genetica* 7:119-169, illus. 1925.

³ SAX, K. THE ASSOCIATION OF SIZE DIFFERENCES WITH SEED-COAT PATTERN AND PIGMENTATION IN *PHASEOLUS VULGARIS*. *Genetica* 8: 552-580. 1923.

⁴ SAX, K. THE NATURE OF SIZE INHERITANCE. *Natl. Acad. Sci. Proc.* 10: 224-227. 1924.

classes and the eyed segregates, because one-fourth of the whites should be homozygous for the extension factor and one-half of the whites should be heterozygous for the extension factor. The white segregates are more or less intermediate in yield, as would be expected, although the differences are not statistically significant. However, the odds are greater than 30 to 1 that the mottled and self classes are more productive than the eyed and white classes.

In calculating the probable errors of the differences in weight or yield of the various classes of segregates the formula $P. E._1^2 + P. E._2^2$ was used, although this method is legitimate only when there is little or no correlation between the two distributions which are being compared.⁵ For instance, it would be possible to obtain a fairly high correlation between the two classes of segregates if the plants were grown on heterogeneous soil. Both classes of segregates would be relatively productive on a good soil and relatively unproductive on a poor soil. By comparing adjacent or neighboring segregates, the degree of soil variability can be determined even when the plants themselves are not systematically distributed so that the method of testing soil heterogeneity suggested by Harris⁶ can be used. In the comparison of the mottled and self segregates versus the eyed and white classes, the yield of each plant was correlated with the yield of the nearest contrasted segregate. This method gives an approximate test of soil heterogeneity, which is measured by the value of r . In this cross the value of r was 0.09 ± 0.07 , indicating that there was no significant correlation between the two classes of segregates under comparison. Accordingly, then, the probable error of a difference, in this case, can be taken as the square root of the sum of the squares of the probable errors of the two frequency distributions.

Evidently the time of flowering is determined by a number of factors, and might be expected to show linkage with one or more of the three seed-coat characters. As indicated in Table 2 the self-colored and eyed segregates bloomed significantly later than either the mottled or white classes. There is, however, no consistent superiority of one class of segregates. High productivity can not be linked with the recessive mottling factors, because most of the eyed segregates are mottled. It is possible that certain combinations working together result in productive or unproductive plants. The odds are about 150 to 1 that either the mottled or the white class blooms earlier than either the self or eyed segregates, but time of flowering does not seem to be linked with any single one of the seed-coat characters under consideration.

The leaf types of the two parents differ considerably and show no evidence of simple segregation in the F_2 . There is not as much association between leaf type and seed-coat characters as might be expected. The leaves of the self-colored segregates are significantly different in shape than the mottled segregates, but when compared with the other two classes of segregates the differences are of doubtful significance.

The association of quantitative characters with vine type is especially interesting. The seed weight of the runner segregate is greater than that of the bush type, although in the parents this relation is

⁵ SAX, K. THE "PROBABLE ERROR" IN HORTICULTURAL EXPERIMENTS. Amer. Soc. Hort. Sci. Proc. (1924) 21: 252-256. [1925.]

⁶ HARRIS, J. A. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. Amer. Nat. 49: 430-454, illus. 1915.

reversed. The relation between seed weight and type of vine may not be due to linkage in the usual sense of the term, but it may be the result of greater vigor of the runner segregate.

The runner segregates in F_2 were more than twice as productive as the bush segregates, although the bush parent was about twice as productive as the runner parent. The runner segregates were also much more productive than the productive bush parent. This reversed relation between yield and parental characters can hardly be explained entirely on the basis of linkage of productivity factors with the indeterminate type of growth. There might, of course, be some such linkage, and yet the runner parent would be relatively unproductive, owing to numerous factors for low yield in other linkage groups. In this case, however, the yield of the F_2 runner segregates was so much greater than the yield of the runner parent that there must be some other explanation for the increased yield in the F_2 . The runner parent was relatively small and weak and was no taller than the Symonds bush parent, a very vigorous, productive type of bush bean. In the F_2 it is probable that the factor for vigor and productivity contributed by the Symonds parent, combined with the runner type of vine which increases the potential bearing surface of the plant, resulted in F_2 segregates of unusual productivity. Although the eyed parent was of the runner type and should have the capacity to carry a large number of seeds, yet it was so lacking in vigor that it produced comparatively few pods.

The differences in date of bloom of the bush and runner segregates are statistically significant and are in accord with the parental relations. It is quite possible that the relation between vine type and time of flowering is due to linkage in the usual sense of the term, although perhaps the greater vigor of the runner type might cause the delay in blossoming. There is no significant difference of runner and bush leaf types in the F_2 . Seed weight and date of bloom show some association or linkage with vine type, but the association is opposite that found in the parents. Such a relation might be expected, occasionally, of course, but it is more probable that the association in this case is due to physiological causes.

SUMMARY

In a cross of Symonds white bean \times a small-eyed bean of local origin, the F_2 segregates gave a ratio of 27 mottled, 9 self-colored, 12 eyed, 16 white, indicating that the simple factors for pigmentation, mottling, and extension of the seed-coat pigment are independent. Vine type, whether runner or bush, is also probably dependent upon a single factor which is independent of the above seed-coat characters. The characters, date of bloom, leaf type, seed weight, and yield per plant, apparently are dependent on multiple factors in each case. If these quantitative characters are dependent on many independent factors for their expression, there should occasionally be some linkage of these characters with the simple qualitative characters of seed coat and vine type. There is some linkage of yield with the extension factor. Date of bloom and leaf type also show some association with certain seed-coat characters, although the relation is not a simple one. Seed weight and

date of bloom show some association or linkage with vine type, but the association is opposite that found in the parents.

The association of yield per plant with vine type is of considerable interest from the standpoint of the plant breeder. In the F_2 the runner segregates are more than twice as productive as the bush segregates, although in the parents this relation is reversed. The runner parent is very weak and unproductive, while the bush parent is vigorous and productive. In the F_2 the combination of vigor and potential productivity with the runner type of vine which permits a maximum development of pods, results in runner segregates more than three times as productive as the runner parent and much more productive than the bush parent.

CRANBERRY FLOWER-BUD INVESTIGATIONS¹

By DONALD SEWALL LACROIX

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INTRODUCTION

The significance of the study of the fruit-bud development in any plant of economic importance is apparent when it is considered that the subsequent fruitfulness of any such plant depends upon the formation and proper development of the buds in their incipient stages. To quote Drinkard,² "If it is possible to ascertain the time of the formation of the fruit buds of our leading fruits, and to trace the development of the buds through every stage of their life history, noting the time of every important change, this information will enable us to attack the still more vital question of the *factors* which influence the formation of fruit buds and are conducive to their proper development."

The study of buds through their early stages is a rather recent phase of horticultural research, the most important of such investigations having been conducted since the late nineties. Fruit growers have recognized for many years that the buds of our important fruits are formed some time during the season previous to blossoming. Pomologists of many of the experiment stations have worked out in detail the life histories of the fruit buds of practically all of the most widely grown fruits, it now being known just when bud formation takes place and when the important changes come about in the subsequent development of the buds.

In 1901 Goff³ reported on a few preliminary studies of the cranberry bud in Wisconsin. Since that time no work has been recorded indicating similar studies of this plant. In view of this and of the fact that cranberry growing is one of the important industries in Massachusetts, it was deemed advisable to investigate and bring together observations on the life cycle of the cranberry blossom.

OUTLINE OF THE WORK

The bud studies were conducted by the author on Massachusetts bogs. It is a general practice in the Cape Cod district to flood the cranberry bogs with fresh water during the winter. This winter flowage may be held on the bogs until late in May, as a control measure against certain insect pests; or it may be drained off early in April. Here a problem presents itself. What effect has early drainage on the development of the cranberry bud and the subsequent development of the fruit, as against late holding of the winter flowage?

¹ Received for publication Jan. 18, 1926; issued August, 1926. Submitted for publication with the approval of the Director of the Massachusetts Agricultural Experiment Station.

² DRINKARD, A. W., JR. FRUIT-BUD FORMATION AND DEVELOPMENT. Va. Agr. Expt. Sta. Ann. Rpt. 1906/10: 159-205, illus. 1911.

³ GOFF, E. S. INVESTIGATION OF FLOWER-BUDS. Wis. Agr. Expt. Sta. Ann. Rpt. 18: 306-310, illus. 1901.

The studies, carried on through three complete seasons, have been divided into two parts: (1) A study of the morphology of terminal buds taken throughout the year; and (2) blossom counts and berry counts in the field. In each case an attempt has been made to note any differences on "early drained" bogs and "late-held" bogs, to determine just what effect early draining or late holding of winter flowage has on the buds, blossoms, and set of fruit.

MORPHOLOGY AND LIFE HISTORY OF THE CRANBERRY BLOSSOM

Material to be used in a study of the morphology and life cycle was collected on the experiment station bog at East Wareham at two-week intervals through summer and fall; at monthly intervals during the winter; at two-week intervals after April first; and semi-weekly immediately before the blossoming period. Samples were taken from each of the two most widely grown varieties, Early Blacks and Howes.

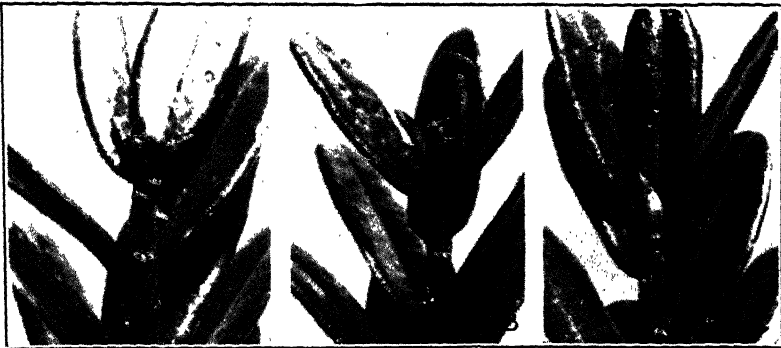


FIG. 1.—Types of cranberry terminal buds: A, Terminal bud containing incipient fruit buds; B, terminal bud of "rosette" type; contains no incipient fruit buds; C, terminal bud which develops into new growth with no fruit buds. All $\times 8.3$

Each sample consisted of 25 terminal buds, and was subjected to the usual process of killing in chromi-acetic acid and washing. The material was then carried through the alcohols to 70 per cent in which it was preserved until winter, when the embedding, sectioning, staining, etc., were completed in the laboratory.

The cranberry vine, as it trails along over the surface of the bog, sends up woody stems or uprights. The stems may or may not bear terminal buds. The terminal buds, when well developed, may be one of these three types: (1) A round, compact, solid bud, covered with tightly fitting scales (fig. 1, A); or (2) a loosely set rosette of scales and small leaves (fig. 1, B); or (3) an elongate, sharp-pointed, scale-covered "shoot bud" (fig. 1, C). The first type contains blossom buds. The second contains no blossom buds, it usually developing as a result of some injury to the normal terminal buds. The third type usually develops into a new growth of upright, and contains no embryo flowers. These three types are usually distinct by the first of October. Prior to that time it may be difficult to distinguish between a terminal bud of the first type and one of the last, since they assume similar shapes early in the season. It is the first type which is dealt with in this paper.

The photomicrograph of the longitudinal section of a terminal bud shown in Figure 2, A, represents the general condition of buds late in July and early in August. The bud in Figure 2, A, was taken August 1, 1924, and it is representative of buds taken during the two previous years on that date. Under the bud scales there is a "crown" or growing point of meristematic tissue. Incipient bud scales are present as small lateral outgrowths. No flower-bud primordia are yet visible.

Figure 2, B, is representative of sections of buds taken in the middle of August, the figure shown being from a bud taken August 15, 1924. An incipient flower bud has started as a minute lateral outpushing, as may be seen at the right of and just below the growing point. The earliest embryo flower bud on the station bog was found on August 10, 1922. Goff⁴ states that he found the first incipient flowers in Wisconsin on September 16.

Figure 2, C, is representative of buds taken from late August to the middle of September. The incipient flower buds have become larger, and lateral projections around the bud on the left are the first intimation of petals. These projections are more pronounced in Figure 2, D, which is typical of buds in middle September and early October. In this bud the rudimentary sepals can be seen immediately outside of and below the developing petals.

In October growth is apparently limited to the petals and sepals, an incurving and overlapping of the immature petals taking place. It is in this condition that the buds pass the winter, and they are thus doubly protected by the bud scales and by the overlapping petals (fig. 2, E).

Growth is resumed soon after the removal of the winter flowage; it is manifested in the terminal bud by the formation of two protuberances beneath the incurved petals (see bud at right in fig. 2, F). It is at this point that the tissues for the future stamens are first laid down as visible thickenings, in the formation of these two protuberances, which are the primordia of the stamens. Similar buds have been taken on late-held bogs where the water was shallow and warm, but as a general rule this type of growth is found only after the winter flowage has been off for a week or more. Up until this time, there has been no marked change in the outward appearance of the terminal bud.

In Figure 2, G (bud taken May 3, 1923) the conditions existing late in April to early in May are shown. Here the small flower buds are no longer sessile but are distinctly stalked, the petals and sepals are more elongate, and the primordia of the stamens are somewhat larger. In the bud at the right a central prominence beneath the immature stamens indicates an incipient stage of the future pistil. A distinct swelling and rounding out of the whole terminal bud has become apparent, and the first indications of "bursting" are visible. The bud scales are no longer wrapped tightly over the bud, but are beginning to separate. Figure 3, A, shows an upright bearing a terminal bud in this stage.

Figure 2, H, which is representative of buds taken in the middle of May, shows clearly (at the left) two of the four or five protuberances which later become elongate and fuse to form the pistil.

⁴ Goff, E. S. Op. cit.

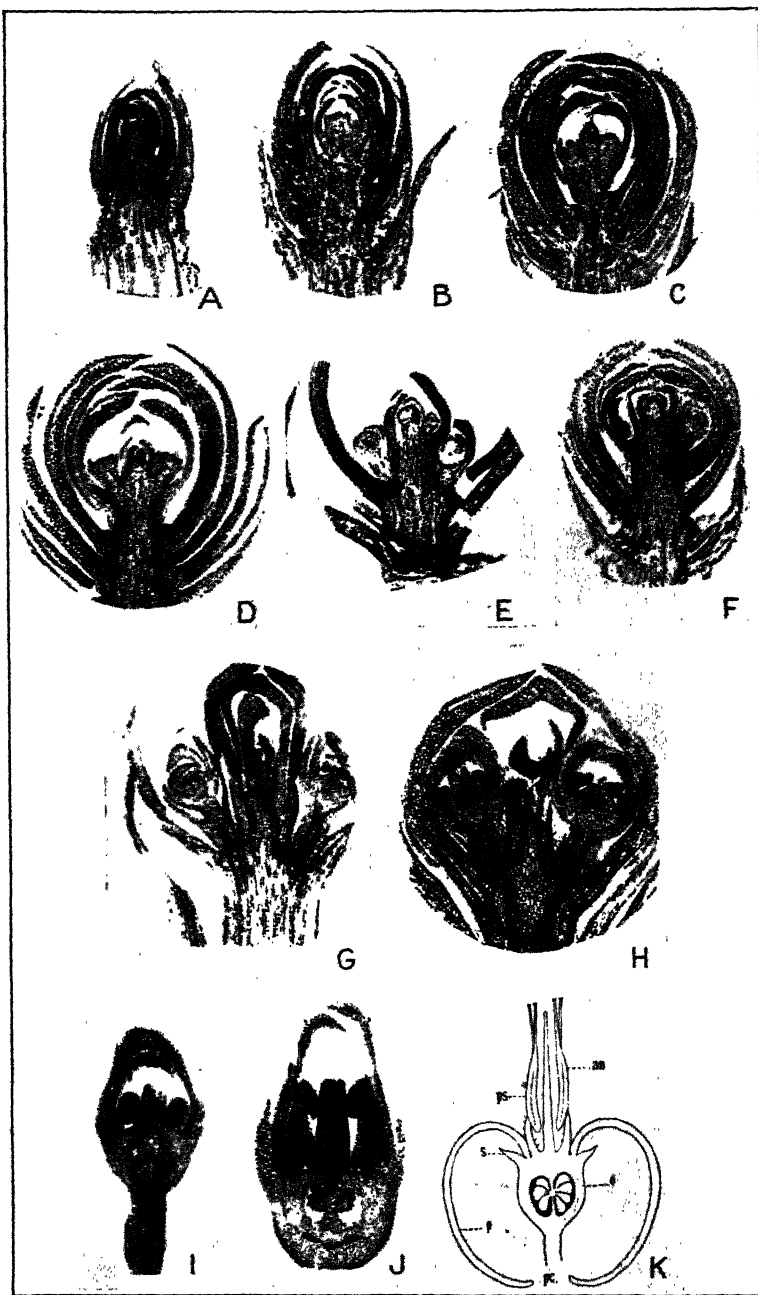


FIG. 2.—Axial longitudinal sections of terminal buds, flower buds, and mature flower, of cranberry, taken at different stages of development: A, Terminal bud taken August 1; B, terminal bud taken August 15; C, terminal bud taken August 20; D, terminal bud taken September 20; E, terminal bud taken October to April; F, terminal bud taken April 17; G, terminal bud taken May 3; H, terminal bud taken May 17; I, terminal flower bud taken May 27; J, terminal flower bud taken June 9; K, diagrammatic sketch of mature flower. (an, anther; ps, pistil; pc, pedicel; s, sepal; p, petal; o, ovary.) (A to J, inclusive, $\times 20$)

Bud conditions during the latter part of May are represented in Figure 2, I (bud taken May 27, 1923). This photomicrograph was taken of an immature flower bud which had become wholly independent of the protective bud scales. It had reached the "rough-neck" stage, so called because the small flower buds are scattered closely along the new shoot, giving the latter a rough appearance (fig. 3, B).

Figure 2, J (a photomicrograph of a bud taken June 9, 1923), represents the general condition of buds shortly before blossoming, a stage that is sometimes referred to as a "pod" (fig. 3, C and D). The immature ovary is distinct, and the seed primordia (ovules) can easily be observed hanging as clusters in the cavity. The fusion of the carpels to form the pistil is well toward completion. The petals are more elongate and are arching upward and outward prior to opening. A slightly more advanced condition is indicated in Figure 3, D, and E.

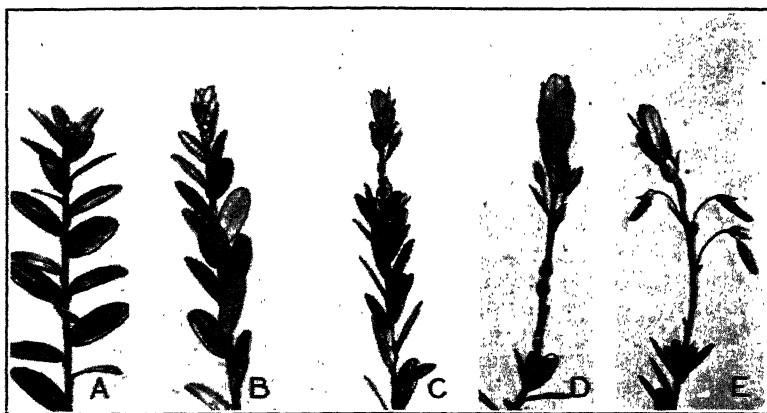


FIG. 3.—Upright, or shoot, of cranberry, showing buds in progressive stages: A, Terminal bud just bursting open; B, an upright in so-called "rough-neck" stage; C and D, flower buds in the "pod" stage; E, flower buds drooping just before opening. (All natural size)

A diagrammatic sketch of the mature cranberry flower in longitudinal section is shown in Figure 2, K.

These illustrations show the complete life cycle of the cranberry flower. In each case an attempt was made to pick out samples of material that were fairly representative of conditions at the various periods. There is more or less overlapping. For example, in some instances buds were found passing the winter in the condition represented in Figure 2, F; but such a case was not a common one and could not well be called a fair sample of conditions in the winter.

FIELD OBSERVATIONS

Many growers have held the opinion that, although late holding of winter flowage on cranberry bogs is beneficial in controlling certain insect pests, such practice has a tendency to reduce flower-bud development and consequently to reduce the yield. As far as can be determined all arguments advanced on either side of the question have not heretofore been substantiated by actual tests. In view of this fact it was considered desirable to collect data on bud conditions from a large number of bogs over a period of several years, and in

this way try to arrive at more definite answers to the various questions raised in connection with late holding of winter flowage.

For three seasons the writer recorded dates of water removal from 16 bogs in southeastern Massachusetts, and classified them into two groups: (1) "Early drained" bogs, or those drained of the winter flowage on or before April 15, and (2) "late-held" bogs, those kept under full winter flowage until May 20 or later.

In the spring of 1923 bud counts were taken on bogs of each group. One hundred terminal buds were collected from each bog in April. These buds were cut transversely and examined with a hand lens to determine whether immature flower buds were present. The study was repeated in May after water removal, and it was concluded that late holding of the winter flowage did have a retarding influence on bud development.

Upon the removal of this flowage late in May, growth is resumed and development advances with great rapidity, the blossoming period being not more than a few days later on late-held bogs than on early drained ones. Buds exposed by late drainage are not subjected to the setbacks of cold weather and heavy frosts of early spring as are the buds exposed by early drainage.

The development of the buds of Howes closely parallels that of the Early Blacks until late spring when the development of the Howes advances less rapidly than does that of the other variety. There may be a difference between the two varieties of from 5 to 10 days in the dates of first blossoms. The incipient flowers in either variety are in evidence by the middle of August.

During the period of bloom, counts were made on these bogs of the number of blossoms per hundred uprights. To do this, 100 uprights were taken at random on each of two ends of a bog, from the two most commonly grown varieties, Early Blacks and Howes. The blossom counts are given in Table 1, the figures being the averages for each season of all early drained and late-held bogs.

TABLE 1.—*Counts of cranberry blossoms and berries on uprights taken at random from bogs, from Early Blacks and Howes varieties, the two most commonly grown in the Cape Cod section*

EARLY DRAINED BOGS						
Year	Number of blossoms per 100 uprights		Number of berries per 100 uprights			
	Howes	Blacks	Howes		Blacks	
			Set	Not set	Set	Not set
1923.....	351.5	414.6	158.0	252.2	163.5	232.5
1924.....	320.4	300.0	135.2	116.0	149.8	127.0
1925.....	281.0	260.0	154.0	135.2	163.0	92.5
Average.....	317.6	324.8	149.0	167.8	159.0	150.6
Per cent set.....			46		51	
LATE-HELD BOGS						
1923.....	304.0	300.8	189.6	150.3	160.4	133.4
1924.....	302.0	248.0	160.8	100.0	123.3	111.3
1925.....	279.0	301.0	162.0	109.6	178.0	121.5
Average.....	295.0	283.2	170.8	119.9	153.9	122.0
Per cent set.....			59		56	

The average for the three seasons indicates that late holding may be responsible for a reduction in the number of flowers. For example, for the three years the average number of flowers per 100 uprights of Howes was 317.6 on early drained bogs and 295.0 on late-held bogs.

Flowers were aborted in some instances. That is, the two leaflets or bracts which always accompany a blossom could be found on some uprights, but no floral development took place, no blossom was formed. This condition was observed in 1923, but in such small numbers that it apparently had no important bearing on the question. No such condition was found in 1924 and 1925. Table 2 indicates the average number of blossoms per 100 uprights, as compared with the average number of aborted blossoms, on the two types of bogs. The figures therein indicate that late holding increases the tendency of blossom abortion.

TABLE 2.—Average number of blossoms and average number of aborted blossoms per hundred of uprights on *Early Blacks* and *Howes*, the two varieties of cranberries most commonly grown in the Cape Cod section, on the two types of bogs

1923	Number of blossoms per 100 uprights			
	Howes		Blacks	
	Aborted	Perfect	Aborted	Perfect
Early drained bogs.....	4.75	351.5	0.66	414.6
Late-held bogs.....	11.00	304.0	10.40	300.8

Counts were made in similar manner of the number of berries which set and of the number of "berryless" pedicels (to indicate the "nonset"). These averages are included in Table 1 also. These data indicate that late holding of winter flowage slightly increased the set of Howes berries and very slightly decreased the set of Early Blacks. On the other hand, the number of berries which did not set is fewer in both varieties on the late-held bogs. By adding the number of berries that set and the number of those that did not a sum is derived which may be called the "possible set." Using this as a basis, the percentage set is readily obtained. Forty-six per cent of the Howes set on early drained bogs, and 59 per cent set on the late held. Fifty-one per cent of the Early Blacks set on early drained bogs, and 56 per cent set on the late held. The percentage set was greater on late-held bogs in both varieties. This indicates that while the actual number of blossoms and the actual number of berries may be decreased by late holding, the percentage of berries which set may be increased.

From a practical standpoint, then, late holding of the winter flowage reduces bloom, but increases the set of fruit. Meeting the requirements of a large number of blossoms is such a heavy drain on the energy of a plant that a greater percentage of the latter do not become fruit. On the other hand, if the number of blossoms is reduced through the influence of external factors (late holding) the plant energy has only to supply the requirements of a few blossoms—hence the increased ability to set fruit.

Further investigations on weather conditions (rainfall, temperature, etc.) resulted in but one satisfactory set of correlations, namely,

temperature and yield. The mean annual temperatures for Cape Cod were obtained from records at the meteorological stations at Middleboro, Plymouth, Hyannis, and Provincetown, by averaging the mean annual temperatures of these four stations. The total crop in barrels for each year was compared with this mean annual temperature. The yield was considerably greater in a year following a relatively warm year, and correspondingly smaller in a year following a cool year. See Figure 4.

For example, 1913 was the warmest year in the period from 1902 to 1919, inclusive, and the crop of 1914 was the largest in the period. The years 1904, 1916, and 1917 were the coolest of the period and the crops for 1905 and 1917 were the smallest.

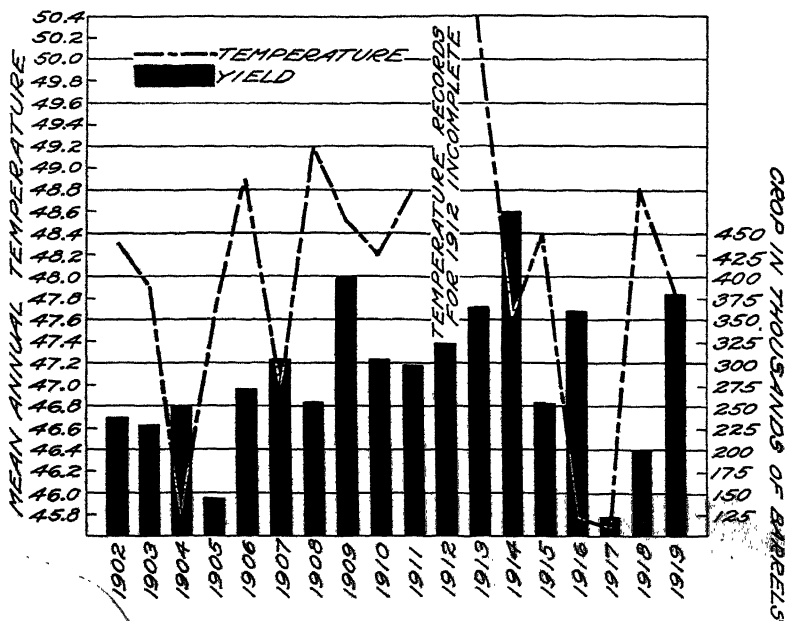


FIG. 4.—Relation between mean annual temperature and the size of the annual cranberry crop, on the Cape Cod, Mass., bogs, for the 18-year period 1902 to 1919, inclusive

It would seem, then, temperature is an important factor in fruit-bud formation. Crops can not be harvested unless fruit buds are formed, and fruit buds apparently require a warm season for the best development.

SUMMARY

Flower buds are first observed on cranberry plants on Massachusetts bogs in the middle of August of the season previous to blossoming.

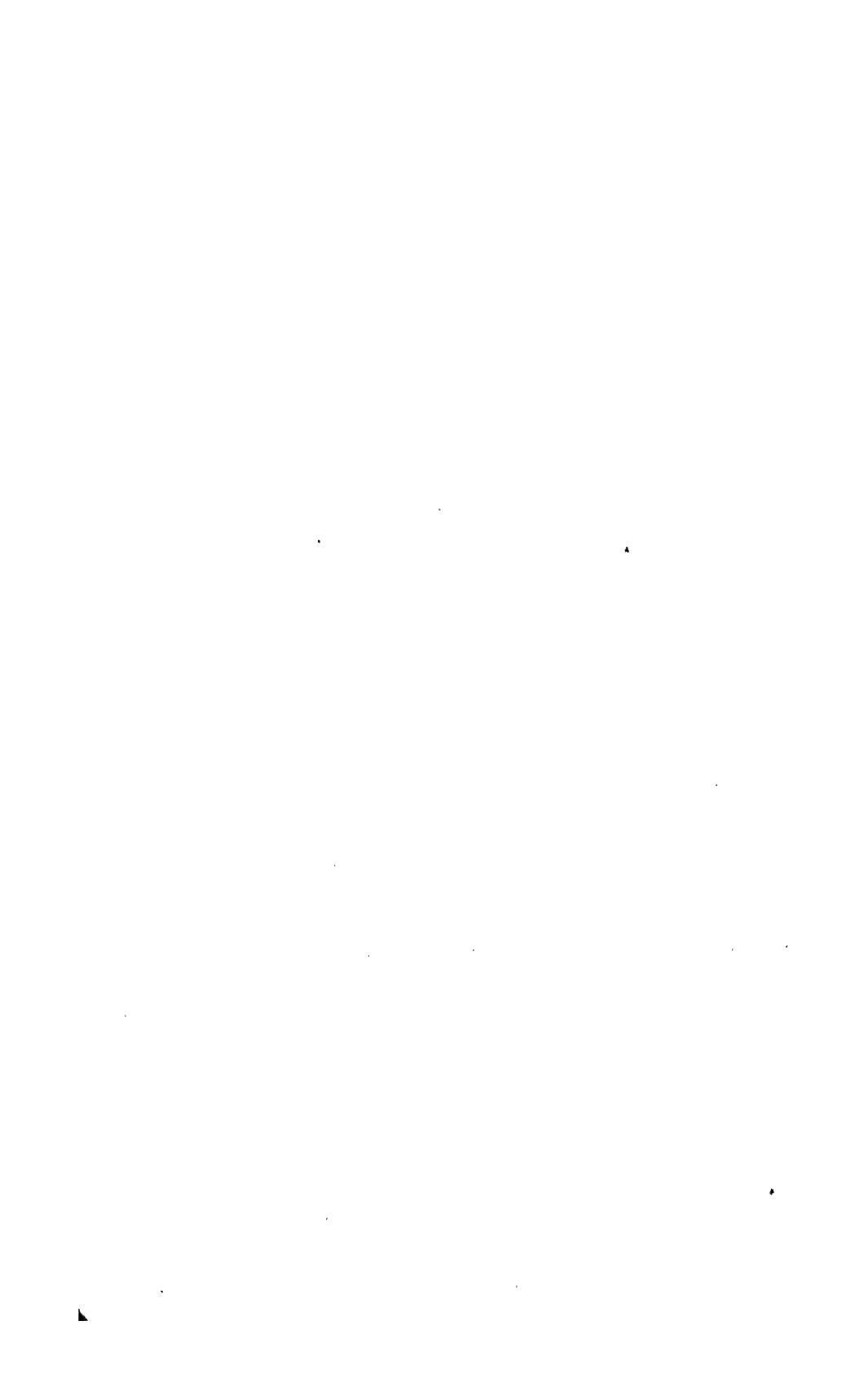
A process of growth and development is observed in the incipient flower buds through the late summer and early fall preceding blossoming.

Apparently no growth that is visible takes place in the buds during the winter.

Growth is resumed in the spring immediately after the removal of winter flowage, and continues at a rapid rate until full bloom in June.

The field observations indicate that late holding of winter flowage tends to retard bud development, decrease the number of blossoms, and increase the percentage set of fruit.

The largest crops of cranberries are harvested the year following a high mean annual temperature, and the smallest crops are harvested the year following a low mean annual temperature. Since the flower buds are formed and partly developed the year previous to blossoming, it appears that temperature is an important factor in the formation and development of the flower buds, a high temperature being favorable to their growth, a low temperature unfavorable.



BROWN-SPOT DISEASE OF SOY BEAN¹

By FREDERICK A. WOLF, *Plant Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*, and S. G. LEHMAN, *Plant Pathologist, North Carolina Agricultural Experiment Station*

INTRODUCTION

The investigations reported in this paper were begun late in the summer of 1922, when the writers' attention was first drawn to a leaf-spot disease of soy bean, *Soja max* (L.) Piper, in North Carolina with which a species of *Septoria* was associated. The crop was at that time practically mature, and considerable defoliation, beginning with the lowermost leaves, was in progress. During the following season the disease was epiphytotic in character, presumably as the result of excessive rains; two protracted rainy periods occurred during that year, one in June and the other in late August and early September, with much damp, warm weather in the interim.

It was evident early in the course of these studies that the disease was very probably identical with one which had first been described in 1915 in Japan by Hemmi² under the appropriate common name of "brown-spot disease." Specimens of this disease were first collected in the Province of Kitamai in September of the previous year by K. Miyabe. Hemmi's report³ shows that it was very common and very widely distributed in 1914 throughout Hokkaido, in the Provinces of Ishikari, Tokachi, and Iburi. It was present that year in every field and was the cause of very considerable damage. In 1915 S. Ito collected it in Honshu in the Province of Echigo. In all probability brown spot has existed in Japan for years, as pointed out by Hemmi, and it occurs in other parts of Asia where soy beans are grown. Miura⁴ observed it in Manchuria, and states that it very probably occurs also in Chosen (Korea). Through the kindness of K. Nakata, Kyushu Imperial University, Fukuoka, Japan, the writers received an unnamed collection made June 18, 1914, by S. Takimoto at Suwon, Korea, which proved to be the brown-spot disease. Brief statements by the writers in the Annual Reports of the North Carolina Agricultural Experiment Station for 1923 and 1924⁵ appear to constitute the only records of the occurrence of this disease within the United States. It no doubt occurs in other States where soy beans are grown, but the only definite information on this point is contained in correspondence with J. F. Adams, who noted it in Delaware in 1923.

¹ Received for publication Jan. 9, 1926; issued August, 1926.

² HEMMI, T. A NEW BROWN-SPOT DISEASE OF THE LEAF OF *GLYCINE HISPIDA* MAXIM. CAUSED BY *SEPTORIA GLYCINES* SP. N. Sapporo Nat. Hist. Soc. Trans. 6:12-17. 1915.

³ HEMMI, T. Op. cit.

⁴ MIURA, M. [DISEASES OF THE MAIN AGRICULTURAL CROPS OF MANCHURIA.] Agr. Expt. Sta. So. Manchuria Ry. Co. Bul. 11, 56 p., illus. [In Japanese. English abstract in Japan. Jour. Bot. 1: (9), 1923.] 1921.

⁵ WOLF, F. A. REPORT OF THE DIVISION OF PLANT PATHOLOGY. N. C. Agr. Expt. Sta. Ann. Rpt. 46: 92. [1923.]

— and LEHMAN, S. G. REPORT OF DIVISION OF PLANT PATHOLOGY. N. C. Agr. Expt. Sta. Ann. Rpt. 47: 83. [1924.]

SYMPTOMS OF BROWN SPOT

The disease is most noticeable upon the foliage, where it is characterized by brown or reddish-brown angular spots 2 mm. in diameter. It is primarily a leaf-spot disease and causes severe defoliation. It appears also on the stems and pods as the plants approach maturity.



FIG. 1.—Seedling soy beans with brown-spot lesions on cotyledons and first true leaves

The first evidence of disease is apparent early in the season, when the first pair of true leaves have formed. At this time there are irregular dark-brown patches, varying in size from minute specks to areas 4 mm. in diameter, on the cotyledons (fig. 1). Before the cotyledons have shriveled and fallen, infections have become apparent

on the unifoliate or first true leaves. The lesions on these leaves are conspicuously reddish brown on both leaf surfaces. They are angular in outline, being limited by the small veins, and vary in size from 1 to 5 mm. in diameter (fig. 2). The larger ones arise from

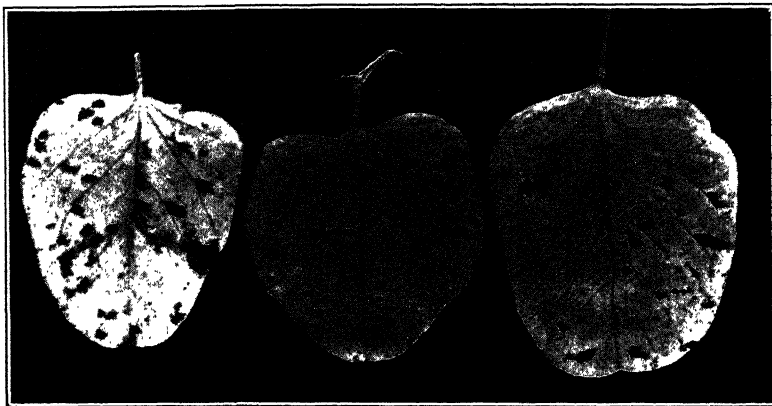


FIG. 2.—Characteristic angular, reddish-brown lesions on unifoliate leaves

coalescence of adjacent spots. The tissues surrounding the diseased areas are pale green at first and then they become decidedly chlorotic, after which the leaves fall off.



FIG. 3.—Mature leaves showing abundance of lesions, chlorosis, and dead leaf tips

The disease next involves the trifoliate leaves, upon which the spots become more numerous as the season advances and, by confluence of the spots, large, light-brown, irregular areas are formed (figs. 3 and 4). The color gradually deepens until the diseased areas are

dark brown to blackish brown, which appears to be intensified with the loss of the normal green of the intervening tissues. The disease progresses upward on the plants from the lower leaves. By late summer, if conditions are favorable for the development and spread of the causal organism, the older leaves become so spotted that it is difficult to distinguish individual lesions. At this time each leaflet

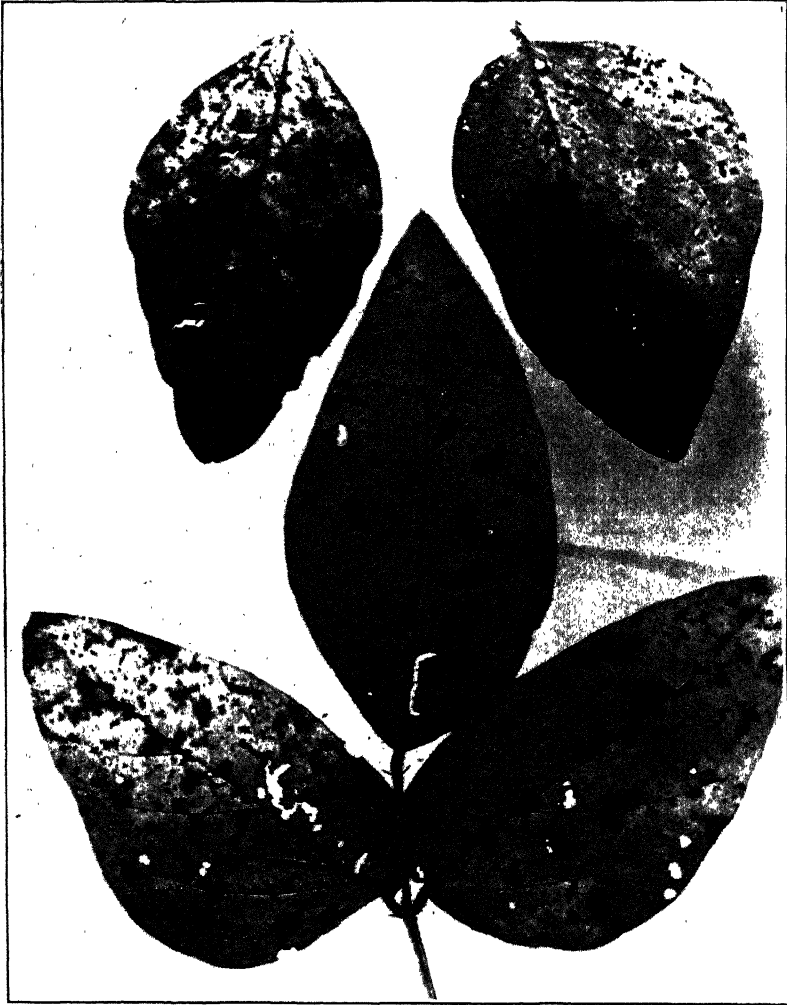


FIG. 4.—A single trifoliate leaf affected with brown spot, and two leaflets with dead tips which would soon have fallen

may bear thousands of specks, with no distinctive microscopic feature except their rusty brown color (fig. 5). Such leaves are prematurely shed from below upward.

The disease on the stems manifests itself by the presence of indefinitely margined, brown discolorations. These lesions vary in size from tiny specks to areas several centimeters in length, and they

may nearly or completely encircle the stems. Tissues adjacent to these discolorations are less green than normal tissues, and as chlorosis advances the diseased areas become more conspicuous (fig. 6). Main stalks, branches, and leaf stalks are all involved in the same manner.

The spots on pods are similar in all respects to those on stems. The smaller areas are pinpointlike, whereas the larger ones may come to involve more than half the surface of the pods (fig. 7).

THE BROWN-SPOT FUNGUS

ISOLATION

Mature lesions on leaves, stems, and pods bear innate, brown pycnidia, and each source has been found to serve equally well as the others in isolation trials. The fungus may readily be isolated when tissues bearing pycnidia are macerated in sterile water and drops of the suspension are spread over the surface of hardened-agar plates. Conidial germination occurs promptly, but it is two or three days before the colonies become visible to the unaided eye. The colonies are olive brown from the first, they develop slowly and remain small. Within three weeks they reach a diameter of 5 to 10 mm. (fig. 8).

The mycelium is dense and stromalike. Pycnidia form in abundance on such media as potato cylinders, potato agar, steamed corn meal, steamed tapioca, and sterile bean pods.

MORPHOLOGY

Lesions on stems and leaves sectioned in paraffin and stained show that the pycnidia are immersed and open to the surface by a large pore (fig. 9, D, E). Those in the leaves are globose to conico-globose,

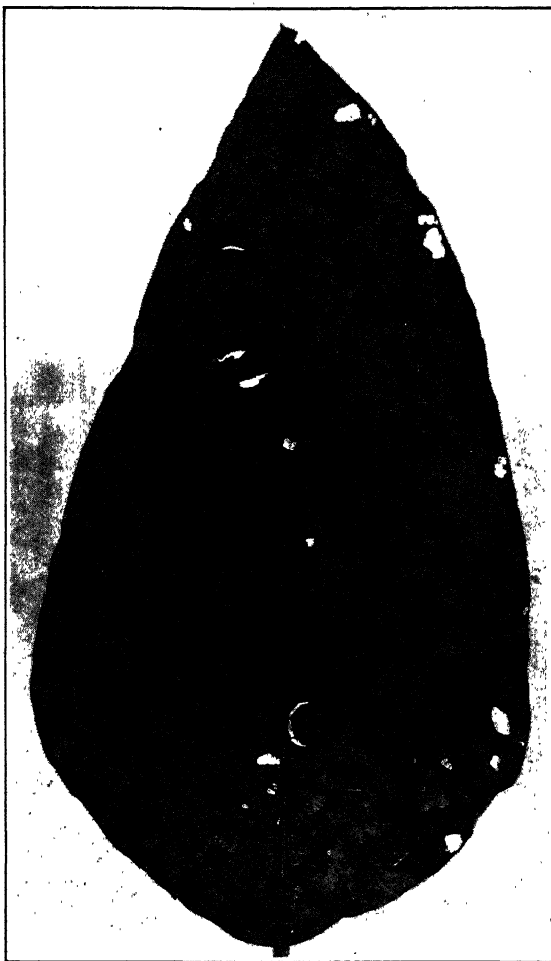


Fig. 5.—A single leaflet in late summer, when the lesions are so abundant that irregular brown patches are formed by confluence of adjacent spots

and open mainly to the upper leaf surface. Those on the stems are flattened, because of their position between mechanical tissues. They commonly measure 90 to 100 μ in diameter, varying from 60 to 125 μ . They are either scattered or quite densely gregarious, and when two are sufficiently close they are flattened on the sides in contact. The pycnidial wall is thin and membranaceous (fig. 9, E).



FIG. 6.—Brown spot on stems and branches

The conidia are hyaline, filiform, curved, and indistinctly 1 to 3 septate. The length varies between extremes of 21 and 50 μ ; but most of them are 35 to 40 μ in length, with diameter varying from 1.4 to 2.1 μ . On germination there is first an increase in size and the septa become more clearly visible because of the constrictions (fig. 9, B). The first germ tubes usually arise at the ends of the conidia, after which one or two hyphae arise from each of the cells. The mycelium is delicate at first, but in mature colonies it is composed of densely branched, interwoven hyphae the cells of which are brown, thick-walled, and beadlike (fig. 9, C).

INOCULATION EXPERIMENTS

The brown-spot fungus appears never to have been proved, by inoculation trials, to be parasitic. The

writers attempted to prove its pathogenicity by use of suspensions of conidia from pure cultures. These suspensions were atomized, in late afternoon, upon healthy plants which had just begun to set seed. Plants growing in beds in the greenhouse were employed in some of the trials, and plants growing out of doors were used in others. When it was found that infections developed so promptly no attempt was made to conserve high degrees of humidity around inoculated plants, and only a few trials were made. In all cases by

the fifth day small, brown discolorations were present in abundance. Characteristic brown-spot lesions had developed a week later, while nearby plants which served as controls remained free from disease. The organism was reisolated from certain of these artificially inoculated plants by use of tissue plantings.

The method of entrance of the fungus was determined by sowing conidia in drops of water on leaves. Then after 16 to 20 hours, by examination in surface view of the epidermis stripped from these leaves, it was observed that the germ tubes enter the stomates (fig. 9, A). As growth continues the hyphae become much branched and ramify between the cells of the leaf tissues.

IDENTITY

The fungus which causes brown spot was compared by Hemmi⁶ with *Septoria soja* Thüm., the only other species known to be parasitic on soy bean, and was found to differ in several essential respects.



FIG. 7.—Soy-bean pods attacked by the brown-spot fungus

He therefore described it as a new species, *Septoria glycines*. The similarity between the pycnidial and conidial measurements of this fungus and those of the fungus with which the writers have been dealing led to the opinion that they were identical. Fortunately it was possible to establish this identity by comparison with Hemmi's type material, collected July 19, 1914, at Tsukisappu, Ishikari, which was sent to the writers. Specimens collected at Raleigh, N. C., were in turn sent to Hemmi for identification. He stated: "I am of the opinion that the fungus on your specimen is macroscopically as well as microscopically the same as my species, *Septoris glycines* Hemmi."⁷

⁶ HEMMI, T. A NEW BROWN-SPOT DISEASE OF THE LEAF OF GLYCINE HISPIDA MAXIM. CAUSED BY SEPTORIA GLYCINES SP. N. Sapporo Nat. Hist. Soc. Trans. 6: 12-17. 1915.

⁷ From a letter dated Mar. 15, 1924, from Takewo Hemmi, Kyo o Imperial University, Kyoto, Japan.

OVERWINTERING AND DISSEMINATION

Diseased leaves and stems from the crop of 1922 and that of 1923 were collected and stored out of doors in unsuccessful attempts to obtain an ascogenous stage. The conidia in such material have been found to survive the winter and be still viable, as shown by germination tests made during March. Refuse from a diseased crop of the

preceding year could therefore be expected to serve as a source of inoculum when the same field is returned to soy beans.

The fact that the pods are subject to attack suggests the probability that brown spot is seed-borne and that contaminated seed are the agency by which it is disseminated. To date, the writers have not been able to demonstrate by cultures and by the aid of a microscope the presence of the fungus within the tissues of the seed or of the conidia on the surface of the seed coats. It has been pointed out, however, that the cotyledons bear lesions. This evidence of seed-borne infection is supplemented by the occurrence of brown spot in a small planting in 1924 from

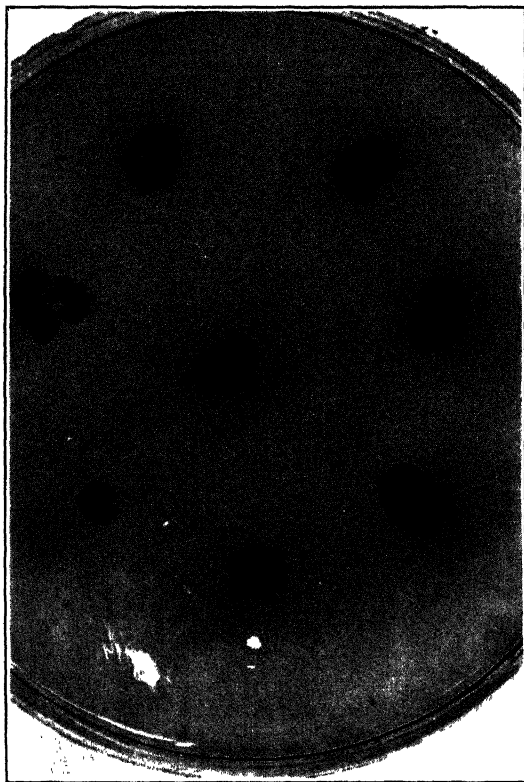


FIG. 8.—Three-weeks-old colonies of *Septoria glycines* on potato agar

seed from a severely diseased crop in 1923. This planting was made on freshly cleared land, and was well isolated from other soybean fields. The presence of brown spot in this field appears to be most reasonably accounted for by its introduction with the seed.

VARIETAL RESISTANCE

The observations on this phase of the problem are very meager and cover only the year 1923, when brown spot was epiphytotic. Just as is the case with other diseases of this crop there are very manifest differences in varietal resistance. Among the varieties grown in the experimental breeding plots which were noted to be severely affected are Black Eyebrow, Virginia No. 12, and a considerable number of hybrids of Virginia. Austin, Wilson Black, Midwest,

and Ito San exhibited only a moderate degree of infection. Mammoth Yellow and Haberlandt 38, the two most commonly grown varieties in North Carolina, were only slightly affected. In this last group belong also Laredo, Biloxi, Lexington, Tokyo, Tar Heel Black, and Chiquita.

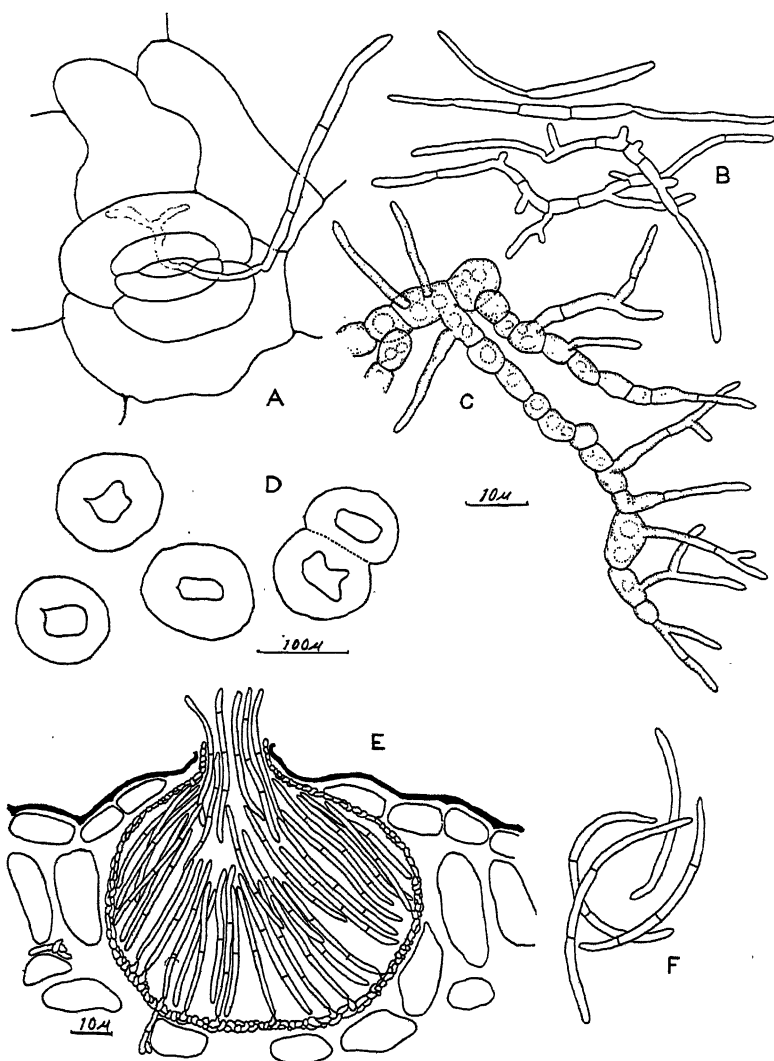


FIG. 9.—A, germination of conidium of *Septoria glycines*, and entrance of the germ tube into the leaf through a stoma; B, germination of conidia of *S. glycines*; C, hyphae from old cultures, with beadlike thick-walled cells; D, surface view of pycnidia of *S. glycines*, showing large ostioli; E, pycnidium in section which opens to the upper leaf surface; F, conidia of *S. glycines*. (A, B, C, and F are drawn to the same scale)

SUMMARY

This investigation deals with a disease of soy beans commonly called "brown spot," first described in Japan in 1915, where it was

collected in several Provinces. It occurs also in Manchuria and Korea, and was first noted in North Carolina in 1922 and in Delaware in 1923.

Brown spot manifests itself by the presence of angular brown or reddish-brown lesions on the foliage. It attacks also the stems and pods.

The disease seems to be seed-borne. It appears first on the cotyledons, spreads thence to the unifoliate leaves, from these to the trifoliate leaves, and causes defoliation from below upward.

Pathogenicity has been established by artificial inoculation. Infection occurs through the stomates, and the mycelium is intercellular.

Brown spot is caused by the imperfect fungus, *Septoria glycines* Hemmi. No ascogenous stage is known. The conidia remain viable during winter on decaying stems and leaves.

PYTHIUM ROOT ROT OF SOY BEAN¹

By S. G. LEHMAN, *Plant Pathologist, North Carolina Agricultural Experiment Station*, and FREDERICK A. WOLF, *Plant Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

So far as the writers are aware, the fungus *Pythium debaryanum* Hesse has never been reported as a parasite of soy bean. During a protracted rainy period in July, 1923, soy-bean plants in the breeding plots at the North Carolina Agricultural Experiment Station were found to be affected by a root-rot disease which was manifestly different from wilt (*Fusarium tracheiphilum* Smith) and sclerotial blight (*Sclerotium Rolfsii* Sacc.), the two root-rot diseases most commonly found on the soy bean in North Carolina. Tissue plantings on acidulated-agar plates were made from root lesions of these diseased plants, and the resultant cultures were identified as those of a *Pythium* of the *debaryanum* type. The results of a study of the morphology of this organism and of its relationship to the disease are recorded in this paper.

Nothing is known of the distribution of this root-rot disease, but since the fungus is cosmopolitan it is to be expected that, under suitable conditions, the disease may occur wherever soy beans are grown. It might appear as a root rot of large plants, as in the case in which it was first noted, and also as the cause of a damping off of seedlings.

THE DISEASE

Attention was first attracted to the disease by the presence of groups of withered and dying plants scattered irregularly throughout the field. The roots and basal portions of the stems of these affected plants were decaying (fig. 1). The lesions normally extended entirely around the stems. Apparently entrance had been effected at the ground level, whence the fungus spread downward and invaded the root system and at the same time extended upward several inches above the ground level, in many instances reaching the lowermost branches.

It has been found that the recently invaded stem tissues are translucent, whereas the older portions of the lesions are brown, necrotic, and involved in a wet rot. In old lesions the less resistant parenchyma is completely disintegrated, but the tougher, fibrous portions remain, the diseased areas assuming a dry, shredded appearance.

When plants are removed from the soil the cortical tissues of the larger roots are found to be so badly disintegrated that they fall away and leave the central woody cylinder exposed. The smaller roots are either completely decayed or break away when the plants are pulled.

¹ Received for publication Jan. 9, 1926; issued August, 1926.

THE FUNGUS

MYCELIUM

The fungus which causes this disease is easily isolated, and it grows well on a variety of media. Potato agar is especially favorable for mycelial development. The tips of young hyphae vary in diameter from 3.5 to 4.5 μ , and are taper pointed. They are finely granular in appearance, but as the hyphae become older the granules enlarge and appear to collect in small clumps. The older hyphae are quite variable in thickness, ranging from 4 to 8 μ .

OOGONIA AND OOSPORES

Two types of reproductive bodies, conidia and oogonia, with their accompanying antheridia, have been observed. True sporangia have not been noted. Oogonia and antheridia are formed sparsely or not at all on potato-dextrose agar. However, if bits of diseased soy-bean tissue are placed in water in a Petri dish and kept at a favorable temperature, oogonia and antheridia are formed in great numbers in three to five days. On this substratum the oogonia are spherical, colorless, smooth, and have a diameter ranging from 16 to 24 μ , with an average of 20.5 μ . In cultures on insects the oogonial dimensions are somewhat greater, ranging from 20.7 to 32.72 μ , with an average of 23.8 μ . Mature oospores grown on vegetable tissue have an average diameter of 17.2 μ ; they are smooth, light brown, thick-wall-

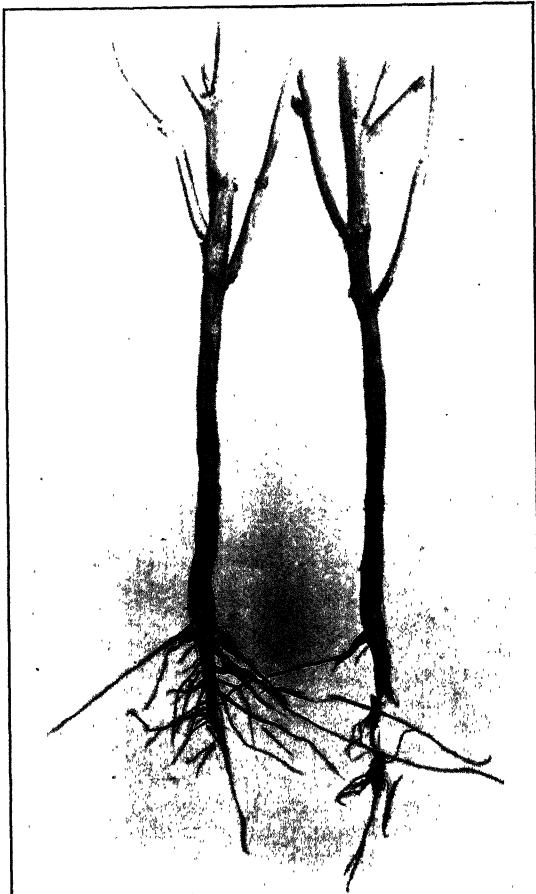


FIG. 1.—Root rot of soy bean caused by *Pythium debaryanum*

ed, and vary in diameter from 12 to 18.5 μ . The protoplasm of the oogonium is at first homogeneous and finely granular.

Initiation of development of the oosphere is marked by a more or less conspicuous clumping of the protoplasmic granules and contraction of the ooplasmic material, leaving a clear periplasmic peripheral zone between the wall of the oogonium and the oosphere. After fertilization, a thin, clear, membrane or vesicle appears about the oosphere, and it thickens and becomes the oospore wall.

ANTHERIDIA

Two types of antheridia, stalk and branch, are developed. In the production of a stalk antheridium, that portion of the oogoniophore immediately subjacent to the oogonium is delimited by the formation of a cross wall. This cell functions as an antheridium and develops a fertilization tube through which the antheridial contents pass into the oosphere (fig. 2, U, V). Branch antheridia arise either as branches of the oogoniophore or of a neighboring hypha. In cases in which the antheridial branch arises from the oogoniophore, the antheridium itself may be supported by a stalk cell or it may be attached directly to the oogoniophore. The branch antheridium is a club-shaped, cylindrical, usually curved cell whose diameter is usually 1.5 to 2.5 times that of its stalk or supporting hypha and whose length is 3 to 5 times its diameter (fig. 2, S). Branch antheridia are not broadly or closely applied to the oogonium, and do not in any case envelop it, but they usually curve in such a way that only their tips are in contact with the oogonium. The protoplasm of both oogonia and antheridia is at first finely granular. When the antheridium comes in contact with the oogonium, the granules in both parts enlarge and collect in clusters, and, in the oogonium, withdraw from the wall leaving a clear margin of periplasm (fig. 2, T). The fertilization tube pushes through the oogonial wall and can be clearly seen to penetrate to the contracted ooplasm. It may have a diameter as great as $2.5\ \mu$. The fertilization tube from the stalk antheridium pushes directly through the septum which separates the antheridium from the oogonium and may easily be observed in situ. Fertilization tubes persist for a considerable time after the formation of the oospore wall. The antheridia persist also after fertilization, but they appear to be empty except for a few scattered granules (fig. 2, R and W).

CONIDIA

Cultures on soy-bean stems in water produced numerous globular bodies which were at first thought to be sporangia. Numerous efforts to induce germination, however, resulted either in the conidial type of germination or no germination at all. Conidia are of two kinds, either terminal or intercalary. Those formed terminally are spherical, while those formed intercalarily have slight polar projections at the positions of the septa which delimit them from their supporting hyphae. They have thin hyaline walls, and are densely filled with protoplasm which contains numerous clusters of fine granules (fig. 2, A to J, inclusive). In size, terminal conidia are approximately equal to ripe oogonia. They will not germinate in the stale water of the cultures in which they have been formed, but germination may become evident within 30 minutes after the conidia are placed in fresh tap water at a suitable temperature (fig. 2, M to Q, inclusive). They germinate well under a cover glass on a slide provided a continuous supply of fresh water is made to flow slowly through the preparation by means of a wick. The germ tube grows rapidly and is filled with clusters of finely granular protoplasm except at the tip where it is homogeneous and clear. As the germ tube elongates, the contents of the spore passes into it and leaves the spore apparently empty. As many as three germ tubes may arise from one conidium.

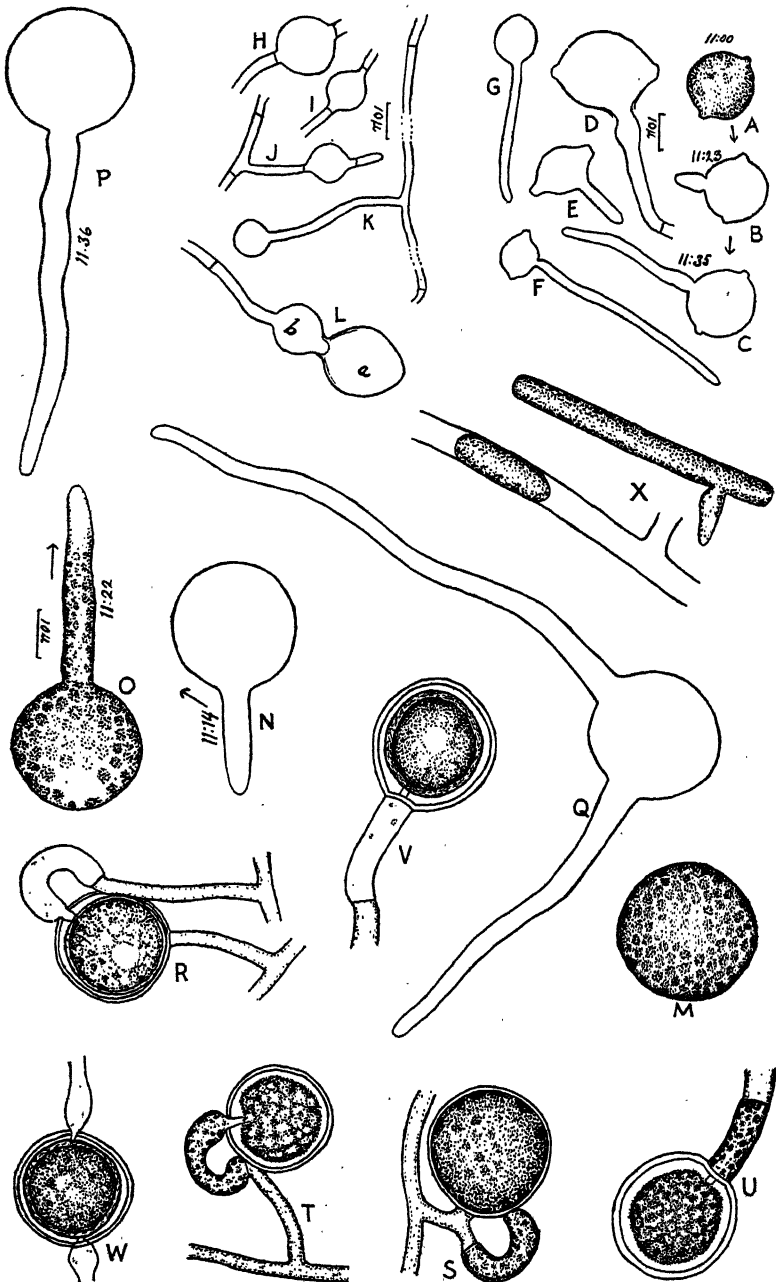


FIG. 2.—The development of *Pythium debaryanum*, the causative fungus in the root rot of soy bean
(For explanatory legend, see p. 379.)

Secondary conidia have also appeared in the cultures (fig. 2, L). Fragments of old hyphae, in cultures a few months old, when placed in fresh water may germinate after the manner of primary or secondary conidia and thus function as propagative bodies (fig. 2, X).

INFECTION EXPERIMENTS

Soy beans about a month old, which were growing in a bed in the greenhouse, were used in the inoculation trials. Cultures were macerated in sterile water and poured on the surface of the ground near the plants. The plants were then shaded with bell jars for 24 hours. Seven days later lesions were present on all of the plants in the area to which the inoculum had been applied. In those cases in which wilting and drooping had begun to be manifest, the cortex was beginning to disintegrate near the ground level in a characteristic manner. In the course of another week a few of the inoculated plants had succumbed, and others bore well-defined lesions on both stems and roots. Plants in the same bed but distant from those in the area to which the inoculum was applied served as controls; and these continued to remain healthy.

The causal organism was reisolated from certain of these artificially inoculated plants and found to be identical with the one used in the inoculation experiments.

TAXONOMY

The character of the mycelium of the soy-bean fungus, the type of conidia, the method of their formation, and the morphology of the sexual organs are clearly those of the genus *Pythium*. The absence of filamentous sporangia and the presence of conidia refer it to the subgenus *Sphaerosporangium* of Butler's² classification, and the smooth oogonia and oospores place it in a group of seven species of which *Pythium debaryanum* is one. In this group *Pythium rostratum*, *P. vexans*, and *P. ultimum* are known to be saprophytes. *P. palmivorum* has pear-shaped sporangia and does not bear conidia, and *P. anguillulae aceti* and *P. intermedium* have catenulate conidia and sporangia. The morphological characters of the soy-bean fungus

² BUTLER, E. J. AN ACCOUNT OF THE GENUS PYTHIUM AND SOME CHYTRIDIACEAE. India Dept. Agr. Mem., Bot. Ser., v. 1, no. 5, 160 p., illus. 1907.

EXPLANATORY LEGEND FOR FIGURE 2

A, B, C, D, E, F, G.—Intercalary conidia germinating in tap water. The rapidity of germination and growth of the germ tube is shown by the time given immediately above A, B, and C.

H, I, J.—Intercalary conidia with supporting hyphae.

K.—A developing conidium, which may become terminal or intercalary.

L.—Conidium (a) that has germinated and formed a secondary conidium (b).

M.—Conidium formed terminally, showing character of its contents.

N, O, P, Q.—Conidia formed terminally, germinating in a continuous stream of water under a cover glass. The rapidity of growth of the germ tube is indicated by the time given alongside N, O, and P.

R.—Oogonium and branch antheridium arising from different hyphae. Fertilization has occurred, as is indicated by empty antheridium and the thin wall about the oospore.

S.—Oogonium with branch antheridium arising from the oogoniophore. No fertilization tube yet evident, nor has the oosphere begun to withdraw from the wall of the oogonium.

T.—Oogonium with branch antheridium arising from the oogoniophore. Oosphere contracted, and fertilization tube in contact with it. Contents of the antheridium have not yet passed into the oosphere.

U, V.—Oogonia with stalk antheridia. Fertilization had occurred in V. The oospore has a thick wall, and finely granular protoplasm with a large oil drop.

W.—Oogonium with two antheridia.

X.—Portions of hyphae filled with dense, finely granular protoplasm. These are resting cells which may serve as propagative structures. The upper one is germinating.

(All figures were drawn with the aid of camera lucida. The magnification of A to L, inclusive, is indicated by the scale shown alongside D and K; that of M to X, inclusive, by the scale shown alongside O.)

make it impossible to regard it as cospecific with either of these six species. In view of its close similarity to *Pythium debaryanum* it is referred to that species. No sporangia such as have been described for this species by de Bary and Butler have been observed by the writers, but instead they have observed numerous conidia which may perhaps be regarded as potential sporangia. This failure to form sporangia and the abundant production of conidia appears to be of common occurrence in strains of this fungus which are referred by various investigators to the species *debaryanum*.

SUMMARY

Soy beans are subject to a root-rot disease which was first observed to be present in North Carolina in 1923.

This malady manifests itself by the presence of necrotic wet-rot lesions, which involve the stems and roots near the ground level and cause the plants to wither and die.

The causal organism accords well in mycelial characters and morphology of conidia, oogonia, and antheridia with *Pythium debaryanum* Hesse, with which it is believed to be identical.

SOY-BEAN ANTHRACNOSE¹

By S. G. LEHMAN, *Plant Pathologist, North Carolina Agricultural Experiment Station*, and FREDERICK A. WOLF, *Plant Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

An anthracnose disease of soy bean, *Soja max* (L.) Piper, was found to be present at the North Carolina Agricultural Experiment Station in 1920, in the plots devoted to investigation of this crop. A brief statement was prepared at that time (7, p. 57)² which is believed by the writers to have recorded for the first time the occurrence of this disease within the United States, and tentatively assigned to the causal organism the name *Glomerella cingulata* (Stonem.) V. Sch. and S. Since then the disease has appeared at the station in succeeding years and has been found in a number of other localities in the State, which has afforded opportunity for a more intensive study of it. It is the present purpose to describe the disease and to record the results of investigations into the morphology and life history of the causal organism.

HISTORICAL

A survey of available literature on soy-bean diseases shows that anthracnose has been noted only in the Orient. Hemmi (2, 3) records the isolation by S. Takimoto of an unnamed species of *Gloeosporium* from diseased soy-bean pods collected in Suigen, Chosen (Korea), in October, 1915. No inoculation experiments were made with this organism, so proof of its parasitism is wanting.

The same investigator isolated in September, 1917, another anthracnose fungus, which was sent to Hemmi (2, 3), and which he identified as *Colletotrichum glycines* Hori. Hori has not published a technical description of this species,³ although its important morphological features, accompanied by appropriate drawings, are given in a report of investigations by Hemmi (2). Neither has this anthracnose fungus been employed in inoculation experiments, but its prevalence on stems and pods at harvest time, together with a knowledge of other species of this genus, was taken by Hemmi to indicate that it was actively parasitic.

DESCRIPTION OF SOY-BEAN ANTHRACNOSE

Soy-bean anthracnose has been observed on the stems and pods, but apparently it does not affect the foliage. Plants in all stages of growth are subject to infection, as shown by field observation and

¹ Received for publication Jan. 9, 1926; issued August, 1926.

² Reference is made by number (italics) to "Literature cited," p. 389.

³ This statement is taken from a letter from Dr. Takewo Hemmi, Kyoto Imperial University, Kyoto, Japan, dated Aug. 24, 1924.

inoculation experiments. The disease reaches its most destructive stage of development in late summer, when the pods are maturing, especially during rainy periods. At this time no definite lesions such as characterize other anthracnose diseases are present, but the affected portions are thickly covered with setose, black acervuli (fig. 1). The macroscopic appearance somewhat resembles the pod and stem blight, *Diaporthe sojae* Lehm., but the fruit bodies of the two may be distinguished with certainty by the use of a hand lens. Moreover, the acervuli are irregularly disposed at the surface of affected stems, with no tendency to arrangement in lines as occurs with the *Phomopsis* stage of the pod and stem blight fungus. Affected plants which are scattered irregularly throughout the field may be readily recognized because of their premature death.

The first evidence of disease is the presence of minute pinpoint-like dark discolorations at the loci of infections, which are visible under favorable conditions four days after inoculation. The surrounding tissues become brown and dead, and by the fusion of individual lesions large areas are formed which may involve the entire surface of the stems and pods. If the pod and pedicel are attacked early enough, seed formation may be partly or entirely inhibited. This may be accomplished by the invasion of the tissues of the pedicel, and in consequence the pods fail to fill, or the mycelium may penetrate the pod wall directly and attack the developing seed. Shrunken seed from such pods invariably yield pure cultures of the anthracnose organism when such seeds are subjected to surface disinfection and plantings are made from seed-coat or cotyledonary tissue.

There is little or no shrinking and collapse of stems because of the large proportion of woody tissue and small proportion of parenchyma.

CAUSAL ORGANISM

ISOLATION

Isolations have been made from diseased stems and pods bearing the conidial stage, from infected seed, and from old, decaying stems bearing the ascogenous stage. Suspensions of conidia in sterile water have been employed in plantings in hardened-agar plates, and this has been found to be a satisfactory method of isolation. Stems bearing the *Glomerella* stage have been placed on bibitory paper in the tops of inverted Petri dishes containing hardened agar. The ascospores are forcibly ejected, lodge on the surface of the agar, and yield pure cultures of the organism by this method. No efforts have been made to make single-spore cultures from either conidia or ascospores, but blocks of agar containing several spores have been transferred to culture tubes. The cultures from all sources, however, have yielded the same type of growth. The mycelium is whitish and rather loose at first, but soon turns dark and the substratum becomes blackened. Within two or three weeks black acervuli bearing pink spore masses and black perithecia have formed. Some strains from conidia have borne conidia alone, even after repeated transfer and cultivation on a variety of media; other strains from ascospores, however, have consistently yielded both the conidial and ascospore stages when cultivated upon the same kind of media.

MORPHOLOGY

As the soy-bean anthracnose fungus was at first thought to be identical with *Glomerella cingulata*, it was grown in parallel cultures with the apple bitter-rot fungus isolated from lesions on apple fruits. The decaying fruits from which these isolations were made

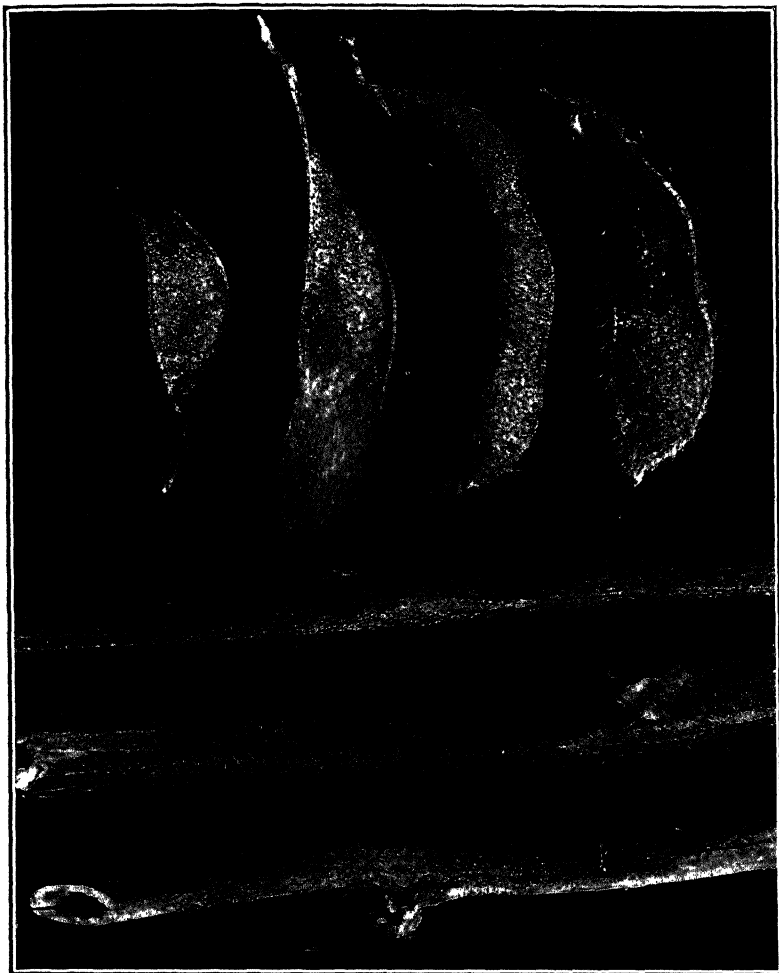


FIG. 1.—Soy-bean anthracnose on pods and stems, showing the distribution of black acervuli

bore at the same time both the conidial and ascogenous stages of the bitter-rot fungus. These in turn fruited in culture with the formation of both stages, so that it was possible to make comparative measurements of the organism from apples and the one from soy beans both from natural sources and from artificial cultures. Table 1 contains a summary of the relative length of ascospores of both organisms from cultures.

TABLE 1.—Relative length of ascospores of the soy-bean anthracnose fungus and the apple bitter-rot organism

[One space=3.75 μ]

Organisms	Number of spores distributed according to length in spaces																Variation in length (μ)	Common length (μ)
	Spaces																	
	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0		
Soy-bean anthracnose fungus.....	0	0	6	27	8	74	23	70	30	17	14	3	3	1	1	2	13. 12-43. 35	18. 75-28. 12
Apple bitter-rot fungus.....	16	62	45	61	24	16	0	0	0	0	0	0	0	0	0	0	9. 37-18. 75	11. 25-15. 00

Comparing the ascospores of the two organisms grown in parallel cultures, it will be noted that those of the soy-bean organism are considerably larger than those of the apple organism, the former varying in length from 13.12 to 43.35 μ , and the latter from 9.37 to 18.75 μ . These differences are more apparent when it is observed that 75 per cent of the ascospores of the bitter-rot fungus range from 3 to 4 spaces, or 11.25 to 15.00 μ in length, whereas 76.6 per cent of those of the soy-bean anthracnose range from 5 to 7 spaces, or 18.75 to 26.25 μ . They range in width from 3.9 to 5.0 μ , and 4 to 6 μ , respectively.

When ascospores are taken directly from decaying host tissue they appear to be more uniform in size, i. e., there are fewer either longer or shorter than the average, than on artificial media. The average measurements accord, however, without regard to the substratum upon which they are grown, and agree well with measurements of the bitter-rot fungus given by Clinton (1) and by Von Schrenk and Spaulding (4). Their extremes range from 12 to 22 by 3.5 to 5 μ .

The asci of the soy-bean fungus vary from 70 to 106 μ in length and from 9.5 to 13.5 μ in width, with an average length of 80 μ and an average width of 12 μ . The asci of the apple bitter-rot fungus vary from 55 to 70 μ in length and from 9 to 10 μ in width, with an average length of 60 μ and an average width of 9 μ . The perithecia of the anthracnose fungus vary from 220 to 340 μ in diameter. The perithecia of the apple bitter-rot fungus vary from 125 to 250 μ in diameter, which measurements accord with those given by Clinton (1) and by Von Schrenk and Spaulding (4).

The Colletotrichum stage of soy-bean anthracnose bears conidia which vary in length from 16 to 25 μ , but the most common size is 20 to 22 μ long by 3.75 to 4.5 μ wide. There are numerous brown setae which vary from 30 to 200 μ in length and from 4 to 6 μ in width. It agrees well with Hemmi's (2) description of *C. glycines*, in which the conidial size is given as 16 to 23 by 3.8 to 4.2 μ , with 15 to 40 setae in each acervulus.

The Gloeosporium stage of the bitter-rot fungus is given by Clinton (1) and by Von Schrenk and Spaulding (4) as having extreme lengths of 10 to 28 μ and extreme widths of 3.5 to 7 μ , with 12 to 16 by 4 to 5 μ as a common size. These measurements agree well with those made by the writers. Measurements of 440 conidia, half of

which were taken from culture on potato-dextrose agar and the other half from a decaying apple, showed an extreme range in length of 6 to 22 μ with 11 to 16 μ as the most common. The conidia grown in culture exhibited a wider range and averaged slightly smaller than those grown on the fruit..

The cultures of soy-bean anthracnose and apple bitter rot exhibit readily distinguishable differences, as shown in Figure 2. Colonies of the former are whitish at first, but soon become smoky black with darkening of the substratum; colonies of the bitter rot are also whitish at first, but become darker (olive tinted) only around the perithecial stromata and acervuli.

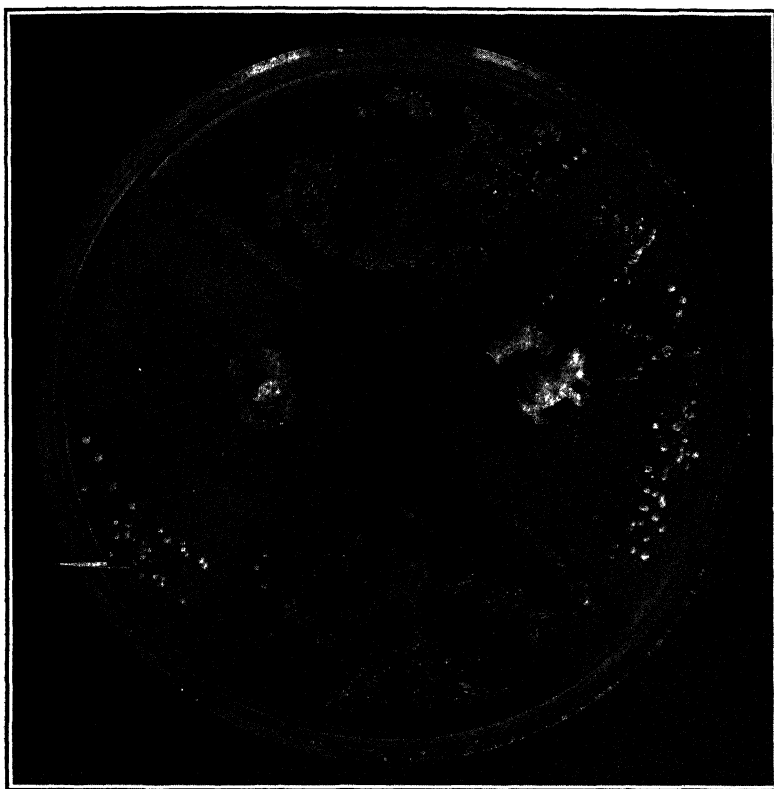


FIG. 2.—The soy-bean anthracnose and apple bitter-rot organism in planted agar plate. The upper and lower quadrants show the bitter-rot fungus, and the right and left quadrants the soy-bean fungus

INOCULATIONS

The inoculation experiments had for their object the determination (1) of pathogenicity of the anthracnose fungus to soy beans, which had not been previously demonstrated, and (2) of the relationship between anthracnose of soy bean and bitter rot of apple. Soy-bean plants, in several series of trials, were inoculated by atomizing them with suspensions of conidia and ascospores from

pure cultures. Certain of the plants were inoculated with the soy-bean fungus, and others with the bitter-rot organism, under identical conditions. Inoculations were made in late afternoon on plants growing in the greenhouse, and the inoculated plants were shaded with newspapers for 24 hours. Each of the series gave uniformly the same results. Within three to four days the first evidences of infection were noted on plants inoculated with the soy-bean organism. Those which were inoculated with apple bitter rot failed to develop any evidence of infection during the entire period in which they were under observation. In another trial three soy-bean plants were inoculated by inserting bits of mycelium and spores into the stems, but here again no infection resulted and the wounds healed promptly.

Reciprocal inoculations were also attempted on several varieties of apples in the following manner: After surface disinfection the fruits were placed in moist chambers and inoculated, on July 2, on opposite sides of the apple. The inoculum, consisting of mycelium and spores from pure culture, was inserted in needle punctures. The tissues surrounding the points of inoculation became involved in decay in the case of all punctures with both organisms. By July 15 the characteristic sunken areas with rings of acervuli had formed in the case of inoculations with bitter rot, whereas the tissues were somewhat darker and less soft in those inoculated with anthracnose of soy bean, and no acervuli had formed on the surface. Furthermore, the characteristic bitter taste was lacking in tissues decayed by the soy-bean fungus.

LIFE HISTORY OF THE FUNGUS

The fungus which causes soy-bean anthracnose has a Colletotrichum or conidial stage and a Glomerella or ascosporic stage. The germination of both types of spores is essentially alike. As an initial step in this process a median septum is generally formed, although many spores remain unicellular. This is followed by the formation of a short germ tube, which is terminated by a brownish appressorium from which the infection tube later arises (fig. 3, F, H). In the inoculation trials in which spores were placed in drops of water on young pods, entrance had been effected within 48 hours by direct penetration of the cell wall (fig. 3, C). The mycelium rapidly extends to adjoining cells and causes their death. It is both inter and intra cellular. Acervuli mature on the lesions in 10 to 14 days.

The fungus passes the winter season either by means of infected seed or of the ascogenous stage. Its occurrence within the tissues of the seed has been established by isolation after surface disinfection, from seed taken from diseased pods. Indirect evidence that anthracnose is seed-borne was obtained by planting seed from affected pods in a situation in which soy beans were not known ever to have been grown, and which was at least half a mile from the nearest soy-bean field. All of the plants in the resultant crop from such seed were seriously affected. In the light of knowledge of other anthracnose diseases it seems reasonable to suppose that the spores which find lodgment on the surface of the seed during harvest could

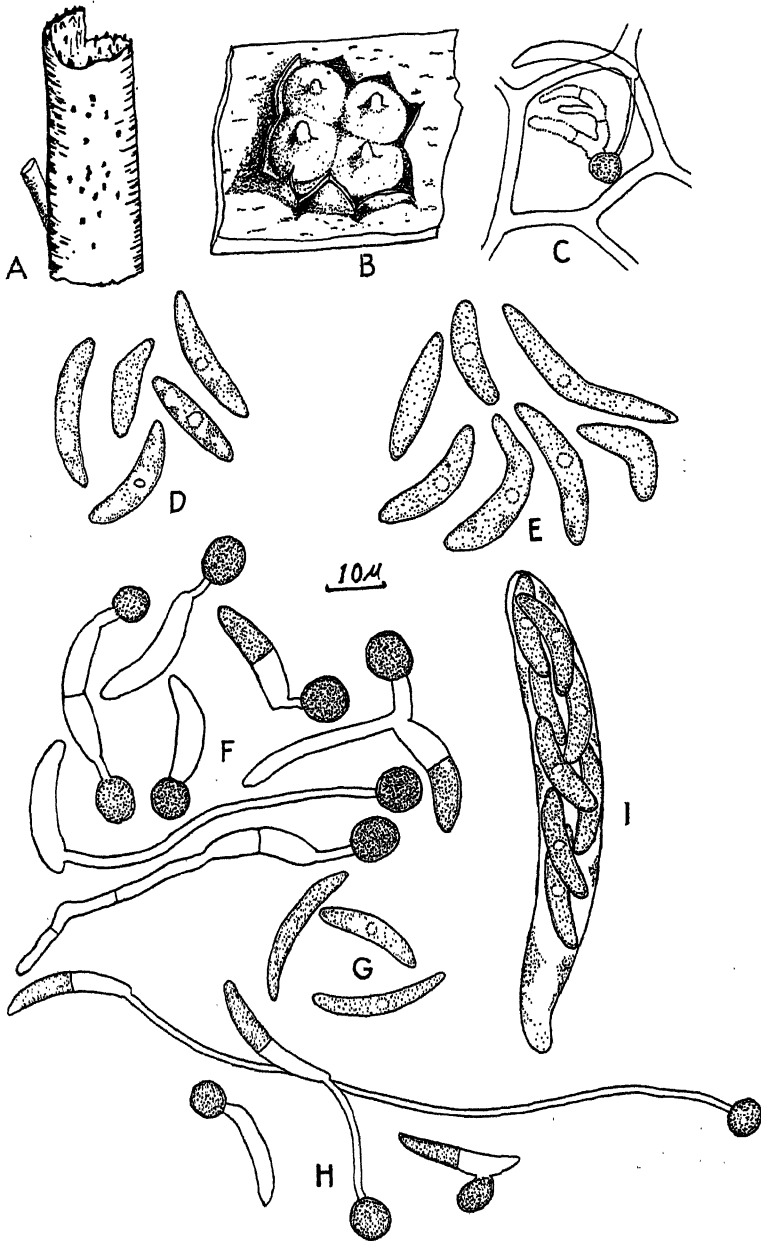


FIG. 3.—A, Diagram of soy-bean stem showing disposition of perithecial stomata; B, diagram of a single stroma bearing four innate perithecia of *Glomerella glycines*; C, germination of conidium and penetration of pod wall by infection tube 48 hours after inoculation, surface view; D, ascospores of *Glomerella glycines* from decaying soy-bean stems; E, ascospores from artificial cultures; F, types of germination of ascospores after 16 hours in water; G, conidia from the Colletotrichum stage; H, germination of conidia with resultant appressoria; I, ascus of *Glomerella glycines*.

also serve as a means of carrying the organism over the winter period and of disseminating it in new fields or localities.

In March, 1924, stems of diseased soy beans from the crop of the previous year which had remained in the field during the winter were found to bear the *Glomerella* stage. The stromata in which the perithecia are embedded arise within the cortex and are at first covered. Each stroma gives rise to one or several perithecia, whose short beaks protrude (fig. 3, A, B). The ascospores are ejected as the asci mature, as previously shown by the isolation trials with inverted agar plates. It is very probable that some of these would come to lodge on plants of the next crop if soy beans were planted in the same field the succeeding year.

IDENTITY OF THE FUNGUS

Plant pathologists would place one or the other of two interpretations upon the foregoing studies on the morphologic and pathogenic characters of the soy-bean anthracnose fungus. Some would regard this fungus as specifically identical with *Glomerella cingulata* from apples. Such an opinion is indicated from Shear's (5) studies on anthracnose of a large number of different plants, in which he concludes that "all are perhaps only slightly specialized physiological forms of one omnivorous species." The same idea is expressed by Taubenhaus (6) from his studies on *Gloeosporium* on sweet pea, in which he states (1) "that the anthracnose disease of the sweet pea is due to the same organism, *Glomerella rufomaculans* (Berk.) Spauld. and Von Sch., that causes the bitter rot of the apple," and (2) "that *Gloeosporium officinale* E. and E., *Gloeosporium gallarum* Ch. Rich., and *Gloeosporium* sp. from May-apple fruit are also the same as *Glomerella rufomaculans*, since they are all able to produce the typical anthracnose disease of the sweet pea and the bitter rot of the pear."

There are others, among whom are the present writers, who would conclude that soy-bean anthracnose is specifically distinct from the apple bitter-rot fungus. The reasons for this may be summarized as follows: (1) The perithecia, asci, ascospores, and conidia of the soy-bean fungus are larger than those of the apple bitter-rot organism; (2) the conidial stage of the former belongs to the form genus *Colletotrichum*, the latter to *Gloeosporium*; (3) when the two are grown on the same substratum the colonies present readily distinguishable differences; (4) the soy-bean organism is pathogenic to soy beans whereas the fungus from decaying apples failed to produce infections on soy beans; (5) the type of decay produced by the fungus from soy beans on apple fruits possesses none of the characteristics associated with apple bitter rot. The fact that the soy-bean fungus causes a decay of apple fruits need not be taken to prove its parasitism, since a mature apple is essentially a culture medium. As is well known, apples can be rotted by a considerable number of fungi not regarded as parasites. More significance certainly should be attached to the fact that the bitter-rot fungus will not attack soy beans than that the anthracnose fungus will produce a rot of apples.

Since it has thus far been impossible to make comparison with specimens from Hori's original collections or with any other collections of *Colletotrichum glycines* from the Orient, the writer does not know with certainty whether the form with which they have been working is identical with *C. glycines*. The descriptive characters mentioned by Hemmi (2) accord sufficiently well, however, to lead to the belief that they are one and the same species. Since the perfect stage is herein appropriately described for the first time, and its relationship to *Colletotrichum glycines* established, a brief technical summary is given, as follows:

Glomerella glycines (Hori.) n. n.

Syn. *Colletotrichum glycines* Hori.

PERITHECIAL STAGE.—Perithecia membranaceous, rostrate, caespitose, 220 to 340 μ in diameter, immersed in a stroma. Asci oblong to bluntly clavate, a paraphysate, 70 to 106 by 9.5 to 13.5 μ ; ascospores hyaline, slightly curved, blunt-pointed, unicellular, 13.12 to 43.35 μ in length, chiefly 18.75 to 28.12 μ , by 4 to 6 μ in width.

Hab.: On decaying stems of *Soja max* (L.) Piper.

CONIDIAL STAGE.—Lesions on stems and pods indefinite in outline. Acervuli black, setae numerous, brown, 100 to 200 μ in length, Conidia hyaline, curved, bluntly tapered, unicellular, 16 to 25 \times 3.75 to 4.5 μ , with 20 to 22 μ as the most common length.

Hab.: Parasitic on stems and pods of *Soja max* (L.) Piper.

SUMMARY

Soy-bean anthracnose affects the stems and pods of this crop and its presence in North Carolina was first observed in 1920. It is believed to be identical with a disease collected in Chosen in 1917 and ascribed to *Colletotrichum glycines* Hori.

The disease is characterized by the presence of numerous black acervuli, uniformly scattered over the surface of the affected parts. It causes premature death of the plants and failure of the pods to fill properly.

The organism is seed-borne, and exists as a mycelium within the seed and as spores adhering to the exterior.

When the soy-bean anthracnose fungus is compared with the fungus which causes apple bitter rot, with which it was at first believed to be identical, it is found to be morphologically distinct, to be of different appearance in culture, and to react differently in reciprocal inoculations.

The ascogenous stage has been found on diseased stems which overwintered in the field, and has been developed in culture. The name *Glomerella glycines* (Hori) n. n. is therefore proposed as a synonym for the conidial stage name *Colletotrichum glycines*.

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DISEASES OF SOY BEANS WHICH OCCUR BOTH IN NORTH CAROLINA AND THE ORIENT¹

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INTRODUCTION

A number of the more important diseases of soy bean, *Soja max* (L.) Piper, have been investigated in the last 10 years at the North Carolina Agricultural Experiment Station (3, 4, 9, 10, 11, 12, 15, 17, 18, 19).² Collections of diseased plants made in various parts of the State have been valuable in interpreting the detailed life-history studies of the causal organisms. The writers' information as to the distribution and prevalence of these diseases has been supplemented by specimens and letters of inquiry from county agents and growers. The most significant result of this work, which has arisen both from the laboratory studies and the field survey, has been the establishment of the fact that certain of these diseases are seed-borne. This fact accounts for the distribution of the diseases throughout the States in which soy beans are grown, serves as a basis for control, and suggests that some of the diseases probably were introduced into the United States with the seed. It is the primary purpose of this report to present such evidence as bears on the last phase of this problem and to give a brief account of the diseases which are common to the Orient and to North Carolina.

As is well known, the soy bean is native to eastern Asia and is a crop of great importance in Japan, Manchuria, Chosen (Korea), and Mongolia. It has been a major crop of the Orient since ancient times, but was of no importance as a farm crop in the United States until about 1880.

In the last 10 years there has been a progressive increase in soy-bean acreage in this country, especially in North Carolina and in several States of the Corn Belt. To-day soy beans are a major crop in the United States.

Apparently the fact that soy beans have only recently become of commercial importance in this country is responsible for the lack of knowledge of soy-bean diseases as found in the United States. A survey of available literature on soy-bean diseases in eastern Asia shows that a similar condition exists there, and that such information as has been recorded on soy-bean diseases in Asia has been contributed largely by Miura (13), Hemmi (6, 7, 8), Takimoto (16), and Nakata. The present writers' knowledge of soy-bean diseases in the Orient is limited by the fact that a considerable part of the work done there has been published in Japanese. Collections of diseased plants and unpublished manuscripts and additional notes sent by Miura, Hemmi, and Nakata have been of invaluable service to the present writers in their work.

¹ Received for publication Jan. 9, 1926; issued August, 1926.

² Reference is made by number (italic) to "Literature cited," p. 395.

WILT

Soy-bean wilt was observed in North Carolina for the first time in 1915. It was ascribed to the same organism which causes cowpea wilt, *Fusarium tracheiphilum* Smith (3, 4). This organism causes a browning or blackening of the xylem of roots and stems, and in advanced stages salmon-colored spore masses, sporodochia, are formed at the surface of the stems and lower branches. The organism is a soil-inhabiting fungus, which enters the plant through the roots. These characters it possesses in common with a considerable number of other *Fusaria*. This disease has been collected at various times in a number of localities in North Carolina, but does not seem to have been noted as involving the uppermost branches and seed pods.

During 1924, however, a serious *Fusarium* disease of the pods was noted in Japan. Specimens collected by T. Nojima at Kitashirakama, Kyoto, were sent to the writers by Hemmi, and the causal organism was found to be indistinguishable morphologically from *Fusarium tracheiphilum*. Another collection was made during the previous year near Kiyomitsu, Sizouka, by K. Hara, of apparently the same organism on diseased pods.

MILDEW

Mildew, when collected in North Carolina for the first time in 1923, was thought to be due to a hitherto undescribed species of *Peronospora*. When it was compared with the species which occur on legumes it was found to be most nearly like *Peronospora trifoliorum* de Bary. However, it differs sufficiently from this species in size of oogonia, of oospores, and of conidia to warrant regarding it as specifically distinct, and so the new name *P. sojae* was proposed (10). While the manuscript which recorded these findings was in press the writers' attention was directed to an account of this disease in Manchuria (13) in which the causal organism was identified as *P. trifoliorum* de Bary var. *Manshurica* Naoumoff (14). Further search revealed that in 1923 Gäumann (5) had raised this variety to specific rank and had accordingly appropriately employed for this mildew the combination *P. manshurica* (Naoumoff) Sydow in lit. When the writers' specimens were compared with collections made by K. Hara in Kanaya, Sizuoka, and by Hemmi in Higashi-Kutchan, Hokkaido, they were found to be identical and to agree morphologically with Gäumann's species *manshurica*. This name has priority, and so *P. sojae* must be regarded as a synonym.

Miura (13) has recorded the presence of mildew in Manchuria. Ocfemia and Weston stated in personal correspondence that they observed it in the Philippines. Butler (1) noted it in India.

Seed from a field in which mildew was abundant in 1923 was planted in an isolated situation the following season in order to obtain evidence of seed transmission of the malady. Mildew appeared on this planting late in June. At first only a few scattered lesions were noted on a few of the plants, but 10 days later the disease was very abundant on all of the plants and the lower surface of the lesions was covered with a violaceous coating of conidiophores. It is believed that in this case the organism was carried on the seed, and that seed-borne conidia served as the source of inoculum.

BROWN-SPOT DISEASE

This leaf-spot disease, which is due to *Septoria glycines* Hemmi, was recognized in North Carolina for the first time in 1922. It was first collected in Japan in the province of Kitamai in 1914; it was epiphytotic that season in the provinces of Ishikari, Tokashi, and Ihuri. In 1915 Hemmi (6) described the disease and its causal organism, and pointed out that the disease had been in Japan for years, and that it probably occurred throughout Asia wherever soy beans were grown. Hemmi sent to the writers specimens collected at Suwon, Chosen. Miura (13) noted it on soy beans throughout Manchuria, and he sent from Kung-Chu-Liug, South Manchuria, specimens of what appears to be the same organism on *Phaseolus munge* var. *radiatus*.

The disease in the Orient has been recorded as a leaf spot on both the primary and trifoliate leaves. It occurs in North Carolina also on stems, pods, and cotyledons, and has been shown to be disseminated by means of contaminated seed (19).

POD-AND-STEM BLIGHT

Pod-and-stem blight (*Diaporthe sojae* Lehm.) was found in North Carolina for the first time in 1920. No reports have come to the writers' attention of its occurrence subsequently in other States. However, in view of the fact that the disease appears on the pods, and the fact that the fungus either destroys the developing ovules or infects the seed, the disease undoubtedly occurs in other parts of the United States. There appears to be no mention of the presence of this disease in eastern Asia. The writers' knowledge of its occurrence there comes from a single collection made September 20, 1923, of the Phomopsis stage from Kiyomitsu, Shizuoka. These specimens were collected by H. Hara and sent by K. Nakata.

Lehman (9), in a comprehensive report of this disease, pointed out that the causal organism overwinters in the pycnidial stage on old diseased stems, and that the perithecial stage had never been found in the field but had been developed in culture. In March, 1924, however, the *Diaporthe* stage was found on stems which had overwintered in the field. Its relationship to the pycnidial stage was established by comparative measurements with the perithecial stage as had been developed in culture and with which it was found to agree, and by isolation from ascospores which when cultured gave rise to pycnidia like those of the Phomopsis stage.

CERCOSPORA LEAF SPOT

In 1918, Miura (13) collected at Tu-men-ling, South Manchuria, a leaf-spot disease which he ascribed to a new species, *Cercospora diazu Miura*. The spots are irregular to circular areas 3 to 6 millimeters in diameter, which are dark brown at first and become light brown with dark brown borders with age. Miura's brief mycological note appears to be the only published account of this disease, but it occurs in Japan, as Hemmi sent specimens collected at Nango, Miyagi, in 1924, and another collection from Shizuoka, Shizuoka, sent by Nakato appears to the writers to be that of this fungus. In September, 1925, one of the present writers (Lehman) found a leaf-spot disease near Moyock, N. C., which proved to be identical in size of

conidiophores and in size and shape of conidia, as illustrated by Miura (13), with *Cercospora daizu*. Nothing is yet known of the means of hibernation and dissemination of this fungus, but this is very probably accomplished by means of contaminated seed.

ANTHRACNOSE

Soy-bean anthracnose (*Glomerella glycines* (Hori) Lehman and Wolf), in its conidial stage on pods, was first collected in Chosen in 1917. It was named *Colletotrichum glycines* Hori, although Hori has not published the results of his studies. Its essential morphological features, however, are recorded by Hemmi (?) and his account contains appropriate drawings of acervuli, conidia, and setae. To date, the writers have not been able to learn of its occurrence in other countries in eastern Asia, nor have they seen specimens from the Orient.

Anthrachnose was found in North Carolina for the first time in the summer of 1920. It was collected on both stems and pods, on which it forms numerous black acervuli which accord well with the descriptive account of *Colletotrichum glycines* by Hemmi (?). The perithecial stage was developed in culture the first season the disease was studied, but was not found in nature until the spring of 1924.

Since the writers' investigations (12) have shown that diseased seed serve as a means for the dissemination of the disease it is to be expected that it will be found to occur in other States where this crop is grown.

BACTERIAL BLIGHT

Several organisms have been found to be associated with bacteriosis of soy beans, as has been briefly discussed in a recent paper (18). The disease to which the name bacterial blight has been applied is caused by two easily distinguishable organisms, *Bacterium glycineum* Coerper, and *Bact. sojae* Wolf. No diagnostic features have yet been noted, however, for differentiating between the diseases which these organisms produce.

The writers recently received from Hemmi specimens of a bacterial disease collected in June, 1914, at Suwon, Chosen, by Takimoto. These specimens are some of those of the collection upon which Takimoto based his investigations (16). The organism which he isolated from this collection was not assigned a name by him but was compared with the published account of *Bacterium glycineum* (2), that of *Bact. sojae* (15, 17), and that of *Pseudomonas glycineum* Nakano, and found to be most like *Bact. sojae*. He further pointed out that the leaf lesions are quite like bacterial blight in America, but that the occurrence of dark, sunken areas on petioles and stems are features not found associated with bacterial blight in North Carolina. In the writers' opinion, Takimoto's specimens present the appearance of characteristic leaf lesions of bacterial blight. In the light of the fact that Takimoto concluded that the organism which he was studying differed from *Bact. sojae* only in the absence of a capsule, in its failure to effect a change in milk, and in its ability to grow in the closed arm of fermentation tubes, differences which might not be manifest if cultures of the organisms from Chosen and from North Carolina were in the hands of one investigator, and that

Takimoto's specimens look like bacterial blight in North Carolina, it is the writers' belief that the diseases are identical.

The writers have not seen the paper by Nakano in which he gives an account of his studies on *Pseudomonas glycineum*, but Hemmi sent them a copy of Nakano's manuscript. Nakano isolated this organism from collections of a leaf-spot disease found in Kiushu, Kumamoto. He compared it with *Bact. phaseoli* E. F. S., *Bact. viciae* Takimoto, and the soy-bean bacterial-blight organism. These studies show that the bacterium is rod-shaped, and motile by means of a single polar flagellum. It forms yellow to orange colonies on culture media, does not liquefy gelatin, does not form acid from sugars, nor does it curdle milk. On the basis of these characters Nakano concluded that it is distinct from the bacterial-blight organism.

GENERAL DISCUSSION

All of the diseases which have been discussed in the foregoing account, except wilt and *Cercospora* leaf spot, have been definitely proved to be seed-borne. They comprise all the known diseases of soy beans in North Carolina which are of economic importance, except two—bacterial pustule (*Pseudomonas phaseoli* var. *sojense* Hedges) and sclerotial blight (*Sclerotium rolfsii*). The former of these also has been found to be disseminated by means of the seed. Neither has been reported, however, from the Orient.

Soy beans in Manchuria are subject to attack by a fungus which has been identified as *Sclerotinia libertiana*. Singularly, this organism occurs on various crops in North Carolina, but it has not been noted to infect soy beans. Aside from these a number of other fungi have been found in Manchuria to be associated with soy-bean diseases, some of the most important of these being *Hypochnus centrifugus* Tul., *Uromyces sojae* (P. Henn) Syd., and *Pleosphaerulina sojaecola* (Massal.) Miura, whose conidial stage is *Phyllosticta sojaecola* Massal.

SUMMARY

It has been found that various diseases of soy beans are transmitted by means of the seed. Because of this fact it was to be expected that these diseases would occur in eastern Asia, where soy bean is native. To date the following diseases are known to be common both to the Orient and to North Carolina: Wilt (*Fusarium tracheiphilum* Smith); mildew (*Peronospora manshurica* (Naoum.) Syd.); brown spot (*Sep-toria glycines* Hemmi); pod-and-stem blight (*Diaporthe sojae* Lehm.); anthracnose (*Glomerella glycines* (Hori.) Lehm. and Wolf); *Cercospora* leaf spot (*Cercospora daizu* Miura); and bacterial blight (*Bacterium sojae* Wolf).

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THE INFLUENCE OF PHYSICAL FACTORS ON THE VIABILITY OF SPORIDIA OF *CRONARTIUM RIBICOLA* FISCHER¹

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INTRODUCTION

For years members of the staff of the Office of Forest Pathology of the Bureau of Plant Industry, United States Department of Agriculture, and others have attempted with varying degrees of success to artificially infect pines with the sporidia of various species of *Cronartium*. Efforts to infect white pines with the sporidia of *Cronartium ribicola* Fischer have met with discouraging lack of success until the last few years. Various investigators (15, 16, 17, 35, 46, 47, 48, 58, 63)³ have scored successes in this direction, but the problem is so complicated that comparatively little is known about the physical conditions necessary for the successful artificial infection of white pines by this fungus. There are three distinct phases of the problem: (1) The factors controlling the formation, germination, and viability of the sporidia; (2) the conditions existing within the white pines at the time of inoculation; and (3) the physical conditions of the surrounding air at the time of inoculation. A preliminary study of the latter phase was made by York and Snell (63), who infected young plants of *Pinus strobus* L. at certain known relative humidities and temperatures of the surrounding air.

In 1923 the problem of studying the sporidia of *Cronartium ribicola* was assigned to the writers, but because of poor material that season's work was preliminary in nature. The work was continued in 1924. This paper presents the data obtained in both years.

HISTORICAL REVIEW

One can hardly appreciate the status of the knowledge on the germination of the teliospores, and of the formation and germination of the sporidia of *Cronartium ribicola*, without a historical survey of the literature on the genus *Cronartium*. Articles on other rust spores are also important as indicating possible methods of investigating the sporidia of *C. ribicola*. Within certain limits, facts which are true of other spore forms are likely to be true of sporidia. The morphology and cytology of the Uredinales in general have been investigated as thoroughly as those of any other fungus group. Yet Colley's paper (18) on the morphology and cytology of *C. ribicola*,

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² The writers are indebted to J. Raymond Hansbrough for assistance in the experiments in 1924, and to Carl Hartley, under whose direction the statistical parts of this article were prepared.

³ Reference is made by number (italic) to "Literature cited," p. 430.

published in 1918, seems to be the most complete study that has been made of any species of *Cronartium*. A consideration of the physiology of the germination of spores shows that in spite of much work there is still great lack of continuity in the results. Ward (61) said in 1902: "It is astonishing how little seems to have been done in ascertaining the conditions of germination of the uredospores." It seems to the writers, however, that the germination of uredospores has been more thoroughly investigated than that of any other form of rust spore. The following brief historical summary is arranged chronologically, to show clearly each investigator's contribution of new knowledge.

Unger (60) gave figures of the telia of *Cronartium asclepiadeum* (Wallr.) Fr., and of early stages of formation of the promycelia by germ tubes from teliospores in the columella.

The Tulasne brothers (59) also studied *Cronartium asclepiadeum*. They found that there was no ostiole in the telial column as was apparently generally supposed at that time. They also illustrated the germination of the terminal teliospores of the column, the 4-celled promycelia, the formation of sporidia by the promycelia, and the germination of the sporidia.

Bary (8) supplemented the Tulasne brothers' results by studies with other genera of the rusts. He made the following significant statements concerning the infection of hosts by the rusts:

"There is no reason for admitting that a predisposition of the host to a certain disease determines its invasion by the parasite. On the contrary, the healthier the plant the more the parasite prospers, provided external conditions are favorable for its vegetation."

Reess (41) noted the formation of secondary sporidia by *Chrysomyxa abietis* (Wallr.) Unger.

Cramer (19) mentioned the breaking up of the promycelium of *Gymnosporangium* into conidiumlike bodies.

Bary (9) made the following statements concerning the forcible ejection of sporidia from the sterigmata of *Aecidium abietinum* Wallr.

"Die Sporidien werden nach ihrer Ausbildung von den Sterigmen abgegliedert und eine kurze Strecke weit weggeschleudert; letzteres wohl durch den gleichen—meinerseits nicht untersuchten—Mechanismus wie die Sporen von *Coprinus*. Die Kraft, mit welcher sie geworfen werden, ist nicht gross."

Farlow (27), in the first detailed American study of a rust, described the breaking up of the promycelia of *Gymnosporangia* into conidiumlike bodies when they are suddenly dried.

Barclay (1), in a study of the new species *Aecidium strobilanthis*, noted that when germination of the teliospores took place at high temperatures, larger sporidia were produced than when germination was slow from the effect of low temperatures. Later (2) he recorded the breaking up of the promycelium into conidiumlike bodies in *Uromyces solidaginis* Niessl and *Caecoma smilacis* Barclay, and the germination of these anomalous conidia. He attributed the breaking up of the promycelia to the absence of air. He also observed (3) the throwing off of the sporidia of *Chrysomyxa himalayense* Barclay from the sterigmata. He noted (4) the breaking up of the promycelia of *Gymnosporangium cunninghamianum* Barclay and the formation of secondary sporidia. He described (5) the method of germinating rust spores by floating them on water, rather than immersing them in it. He also stated (7) that the secondary sporidia formed by *Puccinia*

jasmini-chrysopogonis Barclay are about two-thirds as large as the primary ones, but that those of *Uromyces cunninghamianus* Barclay (5) were of approximately the same size as the primary ones.

Zalewski (64) studied the throwing off of spores by various Hymenomyces. He also stated that the aeciospores of the rusts are thrown off forcibly to a distance of "10-15 mm." This observed distance appears to be an error due to convection air currents.

Kienitz-Gerloff (33) noted germ tubes of teliospores of *Gymnosporangium clavariaeforme* (Jacq.) DC. breaking up into conidium-like bodies, and the production of several germ tubes from one teliospore.

Plowright (38) compiled the knowledge of the British Uredineae. He stated that the longevity of the spores is influenced by the temperature and the dryness of the air, that the sporidia form germ tubes with somewhat pointed tips, and that sporidia may form secondary sporidia if germinated in water.

Carleton (13) studied the influence of various chemicals on the germination of rust spores.

Rauch (39) studied, among other fungi, three different genera of rusts. He found that the germination of freshly collected spores was influenced by ripeness, humidity, and temperature, but not by light. He also observed that a few days of drying lengthened the time necessary for germination, and that aeciospores and uredospores soon lost viability, but that teliospores retained it for several months after drying.

Sappin-Trouffy (43) reviewed the morphology of the Uredinales. For the genus *Cronartium* he gave figures of teliospores germinating at the tip of the telial column; of a teliospore immersed in water germinating and forming a four-celled structure, each cell of which produced a germ tube but no sporidium; and of the formation of secondary sporidia. He thus confirmed the morphological facts reported by earlier investigators.

Eriksson (25, 26) investigated the effect of cooling on the subsequent germination of various rust spores, including aeciospores of *Cronartium ribicola*. He decided that such precooling accelerated and increased germination. He inclined to the idea that spores wet by rain just before using reacted to the cooling, while those which were not so moistened did not. He plainly described germination of the spores by floating them upon the surface of water in relatively large containers, in contrast with the usual drop-culture method.

Ward (62), working with *Puccinia dispersa* Eriksson, found that external and internal physical factors greatly influenced the distribution and persistence of a rust in its uredo stage. He recognized the effect of maturity on the germination of the uredospores, and the capriciousness of their germination, due to unknown causes. He found that uredospores remained viable in diffused sunlight from 30 to 61 days.

Carleton (14), after extended studies of various rusts, especially the grain rusts, stated that rust spores germinated readily in water; that under ordinary conditions the usual amount of growth was reached in an average of 24 hours; and that all growth stopped uniformly at a certain stage, even in the most carefully sterilized cultures.

Blackman (10) investigated the germination of teliospores of the Uredineae and the formation of the sporidia. He brought out clearly the fact that the sporidia are promptly formed in air, but not when the germ tube of the promycelial cell is immersed in water. Later

(11) he concluded that the teliospore is a spore mother cell which undergoes a tetrad division to form the four sporidia.

Klebahn (34) compiled the knowledge of the heteroecious Uredineae, adding much as a result of his own investigations. He clearly stated the fact that the sporidia are forcibly thrown from the sterigmata, but he did not include the genus *Cronartium* among the rusts in which this phenomenon occurs. He also argued that the spores are carried some distance by wind, and gave convincing proof that this actually occurs with certain species of the Uredineae.

Schaffnit (44) investigated the factors controlling the germination of the aeciospores and uredospores of the wheat rusts. He concluded that they mature only when attached to sporophores, and that moisture favors their production and germination.

Maire (36) summarized the known biology of the rusts. He emphasized that telia formed as a result of the decadence of the leaf or that part of the leaf upon which the sori were located. He reported that sporidia were thrown off forcibly, and that convection air currents picked them up and bore them off to indefinite distances; and that the number of sporidia produced and set free at a given center had a direct influence upon the distance that they might be carried and still infect their hosts.

Dietel (21) investigated the discharge of the sporidia of a number of the rusts, including species of *Puccinia*, *Coelosporium*, and *Cronartium*. However, he conducted only one experiment with *Cronartium* and it was unsuccessful, so this genus still remained to be investigated. He found in the other two genera that the sporidia were thrown off.

Robinson (42) studied the effect of external stimuli on the sporidia of *Puccinia malvacearum* Mont. In 12 hours he got good crops of sporidia, which germinated normally in proximity to leaves of numerous species of plants, and abnormally when near a few specific kinds. He found that the germ tubes were negatively heliotropic and were not attracted by small pieces of leaves in the cultures.

Reed and Crabill (40) made a detailed study of the germination of the teliospores of *Gymnosporangium juniperi-virginianae* Schweinitz, giving especial attention to factors which might influence their functions. They found that the teliospores on the outside of the spore tentacle germinated first and shriveled away, exposing those beneath, which germinated in turn. They determined the cardinal temperatures necessary for germination, that the presence of free water was required, and that carbon dioxide inhibited germination. They found that sporidia germinated by producing simple germ tubes or by forming secondary sporidia, which might germinate in the same manner as the primary ones. They observed that secondary sporidia were produced only when the primary sporidia were kept moist constantly from the time of production until germination. They ascertained that the longevity of the sporidia was five or six days in an air-dried condition; that drying was fatal to them, even at relatively low temperatures; and that in direct sunlight they were killed in two to five hours.

Colley (18) made a careful study of the anatomy and cytology of all the spore forms of *Cronartium ribicola*. He found that telial columns arose either from old uredinia or as entirely new and separate entities; that mature columns were aggregations of vertical

rows of mature teliospores; that the terminal spores of the column were the older ones, new ones being added by cell division at the base of the column; that all of the spores of a column might germinate in situ; that germ tubes from spores within the column pushed forth between the overlying rows of teliospores and then produced promycelia in the normal manner; that germ tubes of teliospores soon formed promycelia with four fertile cells, if not shut off from the air by immersion in water, in which case long germ tubes were formed, but no promycelia; that each fertile cell of the promycelium in turn produced a short germ tube or sterigma, from the tip of which a spherical sporidium was abstricted; that the sporidia germinated by producing relatively stout germ tubes; and that secondary sporidia were formed quite commonly.

Doran (23) determined the cardinal temperatures for the germination of the spores of several of the Uredinales, including the aeciospores and uredospores of *Cronartium ribicola*. He also (24) studied the factors controlling the functions of fungous spores in general. He found that mature spores endured a wider range of environmental conditions than did immature spores; that freshly matured spores were more viable than old ones; that the longevity of spores depended upon the conditions under which they were stored; and that crowding of spores in cultures inhibited germination.

Clinton and McCormick (15, 16, 17) studied the artificial infection of young white pines by *Cronartium ribicola*. They found that infection took place through the stomata of the leaves, and they traced the development of the mycelium in the host and its course down the leaf and into the bark.

Tubeuf (58) had previously found that infection of white pines by *Cronartium ribicola* took place through the leaves, but he had not so carefully determined the course of events within the leaves.

York (49, 50, 51)⁴ made preliminary studies on the longevity of the sporidia of *Cronartium ribicola*. His results indicated that they are relatively short-lived and susceptible to external influences. He developed methods for obtaining abundant sporidia at will.

Spaulding (51) summarized the known facts concerning *Cronartium ribicola*, adding much as the results of the investigations by himself and associates.

Taylor (56) estimated the sporidial production of *Cronartium ribicola* in maximum infections on the various species of *Ribes* of the eastern United States.

York and Snell (63) found that sporidia of *Cronartium ribicola* infected young plants of *Pinus strobus* when the temperature was 64 to 65° F. and the relative humidity was 94 to 95 per cent in the inoculation chamber.

Spaulding (52) found teliospores of *Cronartium ribicola* germinating naturally out of doors in winter and producing sporidia in the snow. He also found that teliospores might retain viability until early winter when kept out of doors.

Buller (12) investigated the setting free of the sporidia of many of the Uredinales. He found in all species studied, that a tiny droplet of water formed at the base of the sporidium just at maturity,

YORK, H. H., SNELL, W. H., OVERHOLTS, L. O., TAYLOR, M. W., and RATHBUN-GRAVATT,* A. OBSERVATIONS ON THE SPORES OF *CRONATUM RIBICOLA* FISCHER. [Unpublished manuscript.]

and that the sporidium was then shot off into space. He erred in attributing to Klebahn (34), the first mention of throwing off of sporidia of the rusts as it had been noted by earlier writers (2, 3, 9, 64). He also erred in saying that Dietel (21) definitely proved that the sporidia of the genus *Cronartium* were thrown off from the sterigmata, for Dietel made only one test with a *Cronartium* and it was unsuccessful. There is little doubt that the sporidia of the *Cronartiums* are forcibly thrown off, but apparently this has not yet been proved.

Spaulding and Rathbun-Gravatt (52, 54) studied the factors controlling the activities of the teliospores, and to some extent those of the uredospores, of *Cronartium ribicola*. They found that the teliospores might survive exposure out of doors for about three months; but that the uredospores survived a shorter time, two months after telia were formed upon the same leaves being about the limit for them.

Snell and Rathbun-Gravatt (46) successfully infected old white pines with sporidia of *Cronartium ribicola*, both with and without moist chambers. They conducted experiments only at high relative humidities and rather low temperatures.

This historical review indicates that knowledge of the morphology and cytology of *Cronartium*, particularly *C. ribicola*, is quite complete. While much study has been made of the influence of physical factors on the germination of spores of the Uredinales in general, it can not be said that the present knowledge on this subject is at all complete. There is now only a meager knowledge of the physical factors controlling the functions of the sporidia of any of the rusts. The physiological factors controlling the functions of the sporidia of *Cronartium* had been largely ignored until the problem of the factors controlling infection of pines was attacked.

MATERIAL NECESSARY AND AVAILABLE

Investigations of the type reported in this paper require an enormous number of sporidia, procurable only from a great number of telial columns, which in turn can usually be obtained only from very large numbers of telium-bearing *Ribes* leaves. Such masses of material can be collected only from *Ribes* bushes growing under the best conditions out of doors and subjected to heavy infection naturally. It is necessary to search over large areas to find enough *Ribes* bushes of the native wild species which fulfill all of these conditions. The different species of *Ribes* vary much in the number of telia and sporidia which they produce. Taylor (56) gives a graphic representation of the variation in telial production. The *Ribes* species also vary much in the typical size of leaves, and in percentage of total leaf surface which bears telia. *Ribes nigrum* L. which produces by far the most abundant supply of telia, was the mainstay in these investigations. Two dozen heavily infected leaves of this species often furnished enough material for a group of experiments consisting of 24 slides, the minimum number required for each day. To obtain a similar number of telia from *R. rotundifolium* Michx., a small-leaved species, more than 600 leaves were used. These were infected more heavily than this species usually is, and they were practically the entire season's crop in an especially good spot.

The period of natural production of telia varies with the season, (51) but for the northeastern States it extends from approximately July first until the Ribes leaves fall from the bushes. In 1923 investigations were conducted at Warrensburg, N. Y., which is located in the region of general white pine blister-rust infection, from August 1, when telia were first produced in abundance, until October 10. In many of these tests in 1923 it was necessary to use telia from stored dry leaves. In 1924 the investigations were continued at the same place from August 4 to September 10, when wet weather had germinated so many of the telia that further work was useless. In 1924 the telial material was of good quality.

An effort was made to test sporidia from all possible species of Ribes growing in that part of the United States. Varying amounts of material were collected from eight species—*Ribes americanum* Mill., *R. cynosbati* L.; *R. glandulosum* Grauer; *R. nigrum* L.; *R. odoratum* Wendl.; *R. rotundifolium* Michx.; *R. triste* Pallas; and *R. vulgare* Lam. The number of tests recorded in Table 3 is a fair index of the relative abundance of telia on the different species of Ribes in the eastern Adirondack region in 1923 and 1924.

About 350 series of experiments were made. Each series consisted of 2 to 12 slides bearing sporidia. In 1924 alone data were taken on more than 30,000 sporidia on the undried control slides, on more than 30,000 on the dried control slides, and on more than 64,000 which were exposed to various conditions. The data were based on representative samples of all the sporidia on each slide. The data were taken by moving the slide two or three times, both vertically and horizontally, across the microscope stage. The number of sporidia for 1923 was smaller than that for 1924. It is estimated that about 1,000,000 sporidia were present upon the slides in the two years, and probably this number was only a fraction of the total number produced by the teliospores, as many sporidia were lost in removing the telial columns from the slides.

METHODS

So far as possible sporidia from telia on newly collected Ribes leaves were used, but weather conditions at times made it necessary to use material which had been stored for three or four weeks. While storage of the telial material reduced the amount of telial germination, the sporidia produced by such material appeared to be as viable and vigorous as those from telia on newly collected leaves; that is, stored teliospores did not produce as many sporidia as fresh ones did, but the sporidia that were produced apparently were of maximum vigor.

GERMINATION OF THE TELIA

Various workers (1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 18, 19, 27, 33, 36, 38, 39, 40, 41, 43, 51, 52, 54) have investigated the morphology and, at least incidentally, the physiology of the germination of the teliospores of the Uredinales, but only a relatively few have included any *Cronartiums* in their investigations.

The oldest teliospores in a telial column of *Cronartium ribicola* are the terminal ones, as the length of the column is increased by the formation of new teliospores at its base (18). A newly formed telial column begins to germinate at its tip (43), and germination

proceeds downward as far as mature teliospores have been formed. Preliminary attempts were made to collect dry sporidia on slides by germinating the telia in situ on the *Ribes* leaves in moist chambers. After the proper germination period the leaves were supported over slides so that the mature sporidia could drop to the slides. Too few sporidia were obtained in this manner, so for the purpose of the tests reported in this paper the telial columns were germinated by the so-called "floating" method, which has been described in an earlier paper (54).

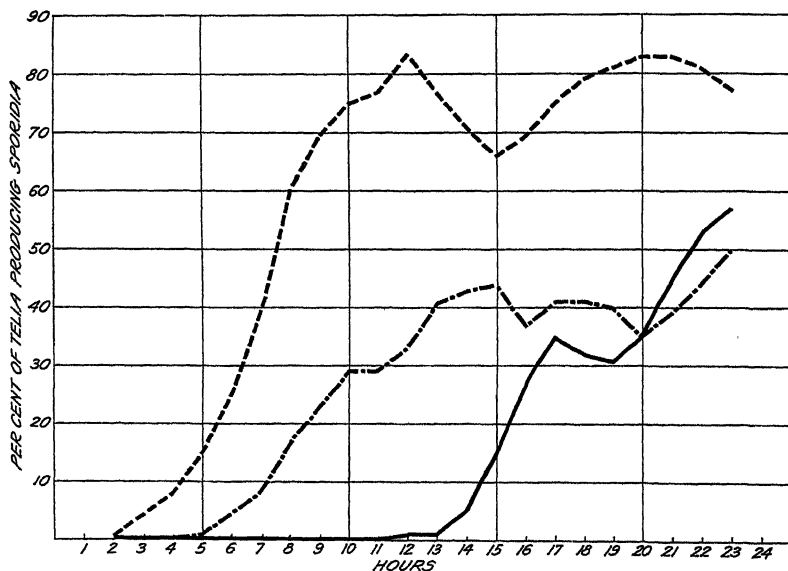


FIG. 1.—Time necessary in preliminary tests for the production of sporidia of *Cronartium ribicola*. The curves (3-hour moving averages) show the number of telial columns producing sporidia at any one time expressed in percentages of the number of columns examined at that time

Dash line: Sporidia produced from telia from *Ribes nigrum* stored 5 days indoors.

Dot-dash line: Sporidia produced from telia from *Ribes americanum* stored 15 days indoors.

Solid line: Sporidia produced from telia from *Ribes nigrum* stored 25 days indoors.

TIME NECESSARY FOR PRODUCTION OF SPORIDIA

Five preliminary tests with material stored for various lengths of time showed that the longer the period of storage the longer the time necessary for germination of teliospores and for production of sporidia. (Table 1 and fig. 1.) Also, the number of viable teliospores tended to decrease with increase in length of time in storage, dry (fig. 1). These facts were determined by hourly counts of germinated teliospores. Upon consideration of the above-mentioned tests, and of others in which hourly counts were not made, it was found that, for stored material, 18 hours might be taken as a standard germination period for obtaining an abundant supply of sporidia without excessive pregermination (germination before the preliminary drying of the sporidia). For fresh material a germination period of 12 hours was sufficient. Several attempts to shorten the germination period by but one hour resulted in scant sporidial production. Like increases in length of the germination period often resulted in increased pregermination. It is of interest to note that Robinson (42) found that

it took 12 hours to get a good crop of sporidia of *Puccinia malvacearum*. It is likely that temperature has much to do with the length of time needed for germination, as York and Snell (63), working at North Conway, N. H., found that only 5 to 6 hours were necessary for the germination of fresh teliospores and the production of sporidia of *Cronartium ribicola*.

TABLE 1.—Number of hours necessary for the formation and germination of sporidia of *Cronartium ribicola*

Ribes species	Collected		Storage period (days)	Hours from start of test to formation of first sporidia	Hours from start of test until number of telia producing sporidia reached the maximum	Hours from start of test to first germinating sporidia
	Place (N. Y.)	Date, 1923				
<i>R. nigrum</i>	Edinburg.....	Sept. 30	5	3	12	11
<i>R. americanum</i>	Warrensburg.....	Sept. 18	15	6	14	15
<i>R. odoratum</i>	Darrowsville.....	Sept. 12	20	6	13	17
<i>R. nigrum</i>	Edinburg.....	Sept. 7	25	13	23	20
<i>R. nigrum</i>	Wadhams.....	Aug. 23	29	8	24	20

PREPARATION OF SLIDES

Since it was impossible to collect a sufficient number of sporidia in a dry condition on glass slides, it was necessary to get them floating in water. After the teliospores had been allowed to germinate for the required time, the telial columns were floated on a clean dry slide. A drop of water was added to the telia on the slide, if it was necessary, and then the telia were stirred around briskly in the water to detach as many of the sporidia as possible from the promycelia. The telial columns were removed with a finely-pointed forceps. Next the fragments of columns were removed under a binocular microscope. The sporidia were then carefully examined for pregermination under a compound microscope. Usually there were few pregerminated sporidia on any slide, and their germ tubes were rarely more than $10\ \mu$ long. In all of the work with sporidia in the two years, only two or three secondary sporidia were found to have been formed as a result of pregermination; they were so rare that they were disregarded. In each group of experiments the amount of pregermination was quite uniform on all of the slides. Careful notes on the amount of pregermination were made for each slide. During the above operations the slides were not allowed to dry.

PRELIMINARY DRYING OF THE SPORIDIA

After the condition of the moist sporidia had been ascertained, they were dried. In the earlier tests this was done out of doors, and in the later ones it was done in a cellar where the atmospheric conditions were nearer the mean of the out door air conditions than those elsewhere. A small electric fan was used to hasten the drying of the slides in the cellar. The time required for drying the slides varied with the temperature and relative humidity, but it was seldom less than 5 and never more than 30 minutes. The time necessary for drying, and the temperature and the relative humidity at which drying occurred, were recorded for every dried slide, except in the pre-

liminary work. As soon as a slide looked dry to the unaided eye it was examined for water under a compound microscope. If no water was detected the slides was considered to be dried.

EXPOSING THE DRIED SPORIDIA

Each slide of dried sporidia to be exposed was subjected to the desired atmospheric conditions, some out of doors, some in the cellar mentioned above, and some in chambers where these conditions were controlled. At the beginning and at the end of the exposure the temperature and relative humidity of the air were recorded. A recording hygrothermograph, which was checked once or twice daily by comparison with a thermometer and a sling psychrometer, gave data for the intervening time. At the end of the period of exposure each exposed slide was examined with a compound microscope for any water which might have condensed upon it. No condensation water was ever found on exposed slides, except in certain tests described below.

GERMINATION OF SPORIDIA

Each series of slides consisted of at least one exposed slide and one unexposed slide which served as a control. This control slide, called the "dried" control, was merely a slide which had received the same preliminary drying as the experimental one, but no subsequent exposure. The majority of the experiments included a second control slide, called the "undried" control, which remained wet throughout the experiment, receiving neither the preliminary drying nor any subsequent exposure. The undried controls showed the viability of the sporidia used in the experiments. The number of exposed slides in a series varied from 1 to 10, according to the factor being investigated. As soon as the telial columns had been removed from the undried control the control was placed in a small germination chamber.

As soon as the preliminary drying was finished the dried control was moistened with a thin film of clean, fresh, tap water. Usually this water film was applied with an atomizer, but in some experiments the dry slides were laid on a flat cake of ice where a very thin condensation film formed almost immediately on them. This method of wetting the dried slides is discussed more fully under the heading "Effect of icing on the viability of sporidia."

After the dried control had been wetted again, it was placed in the same germination chamber as the undried control of the same series. After being exposed and rewet the exposed slide was placed in the same germination chamber with its two controls. Where there were more than one exposed slide in a series, several germination chambers were necessary.

The germination chambers were kept in the above-mentioned cellar, during the germination period, because of the relatively uniform temperature there. Tests showed that no further germination took place in water cultures after about 24 hours. This agrees with Parleton's (14) general statement concerning the germination period of rust spores. It was also found that the germ tubes did not increase in length after about the same period of time. These facts made it possible to examine all of the slide of a series at the same time without introducing serious errors.

Massing of the sporidia sometimes resulted in no germination within the masses. This probably was an oxygen reaction, but it may have been a toxic one. Lack of germination was also noted when telial columns were badly massed (54), and it is apparently quite common when rust spores are massed (24).

EXAMINATION OF THE GERMINATED SPORIDIA FOR RESULTS

At the end of the germination period the sporidia were examined for germination under a compound microscope. Records were made of the number of sporidia counted, the number germinating, the length of germ tubes formed, and the presence of secondary sporidia. The primary and secondary sporidia were of the same approximate

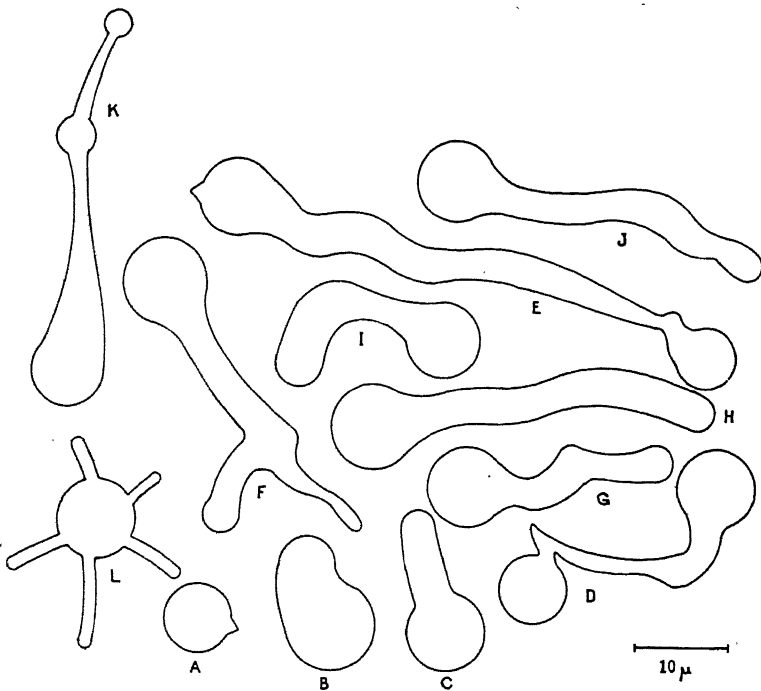


FIG. 2.—Germination of sporidia of *Cronartium ribicola*: A, ungerminated sporidium; B, C, Slight germination of the type referred to as "pregermination" in this paper; D, E, Secondary sporidia; F, branched germ tube; G, H, I, J, vigorous germination; K, tertiary sporidium produced by a secondary sporidium; L, sporidium with several abnormally slender and rather short germ tubes

size, and they resembled each other so closely in every way that the writers could not distinguish one from the other. The presence of secondary sporidia in large numbers often decreased the apparent germination percentages, because many of them were detached in placing the cover glasses before examination. Since these secondary sporidia had not begun to germinate, they increased the number of ungerminated sporidia counted. Germ tubes of primary sporidia which have produced secondary sporidia are likely to be sharply pointed (16, 18). In some slides the number of these pointed germ tubes nearly equalled the number of ungerminated sporidia found. Figure 2 shows some of the various forms of germ tubes produced.

The germ tubes produced by the sporidia in the tests ranged in length from 10 to 100 μ . Germ tubes 50 to 60 μ in length were not uncommon. Vigorous germination yielded germ tubes 25 μ or more in length. The lengths of the germ tubes were important, because they made it possible to distinguish between growth which had occurred before the sporidia were subjected to the preliminary drying and growth which occurred after that time. The presence of secondary sporidia was also important in this connection, because they were practically never found *in situ* as a result of pregermination. Their presence in numbers subsequent to this stage of the experiments could be due only to their formation after the preliminary drying and the exposure of the sporidia.

CORRELATING THE DATA

There was little time in the field laboratory to correlate the results, as all efforts were bent upon clearing up certain practical questions concerning the behavior of these erratic, and, in some respects, very easily affected spores. When the data were analyzed it was found that many gaps still existed. No excuse is offered for this, because all possible data were taken. Since further work upon this problem is deemed inadvisable at present, it is believed that the data should be published so as to be available to future investigators. The results are presented largely by means of graphs, because of the great condensation which is possible by this method of presentation. Publication of detailed tables would be out of the question.

While preparing the data, it was found that although sporidia were very resistant to some adverse conditions, they were at times very sensitive to minor changes in environment. For this reason, the results from a number of early experiments, in which all of the sporidia on both the control and exposed slides had succumbed to some obscure and undetected condition, were discarded.

As stated earlier, the air temperature and relative humidity were recorded for each exposed slide in every experiment. However, the same relative humidity at different temperatures may result from different actual amounts of atmospheric moisture. Saturation deficit, which can be computed from temperature and relative humidity, is an index of the drying power of the air. The writers' data of air temperatures and relative humidities have been converted into saturation deficits in millimeters, in order to reduce these two different variables to a common basis. Saturation deficit can be computed easily by means of meteorological tables (45) and a formula given by Hann (29). Or, relative humidity and temperature can be converted directly into saturation deficit by one reading from a graph published by Meyer (37). Both methods gave the same results.

After the saturation deficits had been calculated, the data were arranged in groups or classes according to the saturation deficit of the air to which the sporidia were exposed. Usually the air conditions remained within one saturation-deficit class during the whole exposure period for each slide. In those cases where the saturation deficit varied from one class to another, the data were grouped in the class of the greatest saturation deficit occurring during the exposure.

Throughout this paper the term "weighted three-point moving average" is used to indicate a moving average each point of which

is weighted by the number of tests made at that point and by the number of tests made at the points on either side. For example, suppose 10 tests with an average of 70 per cent were made at a saturation deficit of 2, 5 tests with an average of 80 per cent at a saturation deficit of 3, and 20 tests with an average of 60 per cent at a saturation deficit of 4. The value for saturation deficit 3 in the weighted three-point moving average would be

$$\frac{(10 \times 70 \text{ per cent}) + (5 \times 80 \text{ per cent}) + (20 \times 60 \text{ per cent})}{10 + 5 + 20} = 66 \text{ per cent.}$$

The value of each point in the moving average was determined in this way.

EFFECT OF DRYING ON THE VIABILITY OF SPORIDIA

Unless it germinates immediately after maturing, practically every sporidium produced in the fields and forests of this country is subjected at some time to some degree of desiccation. Buller (12) and others (9, 21, 34, 36) found that the sporidia of many of the Uredinales are thrown forcibly from the sterigmata. Buller also observed that at the time of maturity each sporidium of some genera of the rusts has at its base a tiny droplet of water and that when the sporidium is thrown off the water drop goes with it. This is a most remarkable provision against desiccation. Unfortunately no species of *Cronartium* has yet been carefully examined to determine whether it is like other genera of the Uredinales in these respects. It is likely that it is, however. Since the susceptibility of sporidia to desiccation may be decisive in controlling the amount of infection which they may cause in white pines, the effect of drying on their viability is very important from a practical standpoint. This problem was one of the most urgent of all those connected with the physical factors controlling the functions of the sporidia of *Cronartium ribicola*. It was attacked from several different angles.

Throughout the present investigation the sporidia were studied on glass slides. Heald and Studhalter (31), working with the spores of *Endothia parasitica* (Murrill) A. and A., found "that conditions of desiccation on a glass surface are more severe than on surfaces on which spores would be dried under natural conditions in the field." They concluded that tests made from spores on glass surfaces did not give the maximum longevity of either ascospores or pycnosporos of *E. parasitica*. In view of these results there is a possibility that sporidia of *Cronartium ribicola*, if they had been dried on some other surface besides glass, would have been even more tolerant of drying than they appeared in any of the reported tests. No comparative tests were made to determine this point, because time was limited. Some longevity tests of sporidia dried on epidermis of *Ribes* leaves or of pine needles and on pine bark are very desirable.

EFFECT OF THE PRELIMINARY DRYING ON THE VIABILITY OF THE SPORIDIA

Since large numbers of sporidia could be obtained only in a wet condition, it was imperative to determine the effect of the preliminary drying to which all sporidia to be exposed were subjected before any experiment could be started. For this reason every experiment consisted of a series of slides of sporidia. Every series contained

an exposed slide or slides, and a dried control slide; and the majority of them also included an undried control slide which represented the vigor of the sporidia before any drying took place. The effect of the preliminary drying has been considered from various angles.

In Figure 3, the percentage of germination of the sporidia on control slides dried at saturation deficits up to 5 mm. are compared with those of the directly comparable undried controls. Inspection of the graph indicates that, on the whole, the undried sporidia germinated better than the dried ones.

The average of the percentages of germinations for the undried control slides was 61.1, while that for the strictly comparable dried controls was 42.7, a difference of 18.4 per cent in favor of the undried ones. When all the experimental data (including those from slides which were not directly comparable) were utilized, the results were practically the same as when only strictly comparable data were used.

The weighted averages of the results in the strictly comparable experiments were obtained as follows: In the undried controls 25,743 sporidia were counted, of which 15,999, or 62.1 per cent, germinated;

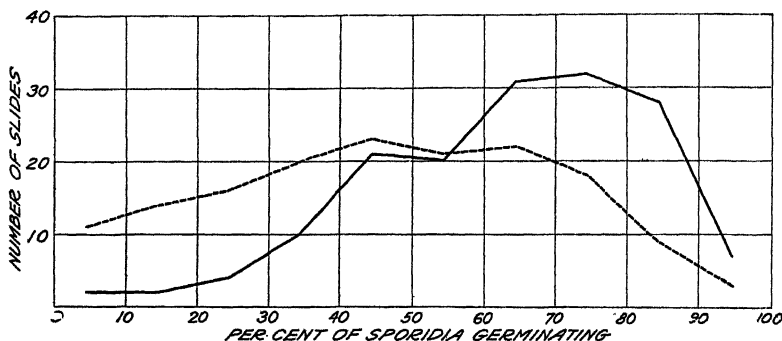


FIG. 3.—Distribution of the germination percentages of sporidia of *Cronartium ribicola* in the controls dried at saturation deficits up to 5 mm., and the distribution in the directly comparable undried controls
Solid line: Undried controls.
Dash line: Dried controls.

in the strictly comparable dried controls, 26,027 sporidia were counted, of which 11,530, or 44.3 per cent, germinated. The difference between the two was 17.8 per cent. When all of the experimental data (including those from slides which were not strictly comparable) were used, the results were practically the same.

Both of these methods of averaging indicate that the preliminary drying reduced the average germination by approximately 18 per cent, on the basis of 100 per cent possible germination. On the basis of the germination actually occurring in the cultures, it reduced germination about 29 per cent.

While the difference between the averages for the dried and undried sporidia is large, the high variability shown in Figure 3 makes desirable some further examination of the significance of this difference. In the pairs of directly comparable slides the undried slides not infrequently gave lower percentages of germination than did the dried ones. However, it is evident from Table 2 that in every one of the 14 groups into which the tests were divided, the percentage of germination on the undried slide was greater than that on the dried

one in more than half of the pairs of slides. Figure 4 gives further information on the relative constancy of the superiority of the undried slides. The results are so consistent as to leave no doubt concerning the general harmful effect of the preliminary drying upon the viability of the sporidia.



FIG. 4.—Comparison of the germination of sporidia of *Cronartium ribicola* in the controls dried at various saturation deficits with that in the strictly comparable undried controls. The figure at each point represents the number of experiments upon which each point is based. This number is the same for both the undried and the dried controls

Solid line: Germination in the dried controls.

Dash line: Weighted 3-point moving average of the same results.

Circle-solid line: Germination in the undried controls.

Circle-dash line: Weighted 3-point moving average of the same results.

TABLE 2.—Relation between the injury caused by the preliminary drying of the sporidia of *Cronartium ribicola* and the saturation deficits at which the drying was done

Ribes hosts of telia from which sporidia came	Number of comparable pairs of slides in which the dried one was subjected to a saturation deficit of—				Percentage of comparable pairs of slides in which undried ones germinated better than the dried ones when the latter were subjected to a saturation deficit of—			
	0-2.9 mm.	3-4.9 mm.	5-6.9 mm.	7-10.0 mm.	0-2.9 mm.	3-4.9 mm.	5-6.9 mm.	7-10.0 mm.
<i>R. nigrum</i>	83	23	8	7	68	78	100	100
Other species besides <i>R. nigrum</i> (the five following).....	30	18	2	—	77	89	100	—
<i>R. cynosbati</i>	5	—	2	—	80	—	—	—
<i>R. glandulosum</i>	13	4	1	—	69	100	100	—
<i>R. odoratum</i>	—	5	—	—	—	80	—	—
<i>R. rotundifolium</i>	10	7	1	—	80	88	100	—
<i>R. triste</i>	2	2	—	—	100	100	—	—

In Table 2 the difference in favor of the undried sporidia was more consistent at the higher saturation deficits than at the lower ones. Figure 4 shows clearly that the magnitude of the difference in percentages of germination in favor of the undried sporidia increased steadily with increase of the saturation deficit. Because the number of experiments at some of the greater saturation deficits was small, the moving average shown by the broken line is the best basis for comparison. The material used in the experiments at the greater saturation deficits happened to be poorer, on the whole, than that used at the smaller saturation deficits. As a consequence of the cramping of percentages near the end of the scale, it is reasonably certain that the differences between dried and undried sporidia in the experiments at the greater saturation deficits would have been even larger than it was if the material had been as good as that used in the experiments at smaller saturation deficits.

Comparison of the results with sporidia from different species of *Ribes* in Table 2 makes it appear that the preliminary drying caused less damage to sporidia from the very congenial host *Ribes nigrum* than to sporidia from less congenial hosts. Comparison of the results from sporidia produced on *R. nigrum* with the results from sporidia from all of the other species of *Ribes* (given in the next line below *R. nigrum* in Table 2) shows that in both of the lower saturation-deficit groups the difference in favor of the undried sporidia was about 10 per cent greater for other species than for *R. nigrum*. In each column, however, the difference between the percentages for sporidia from the two sources was no greater than the standard error, and the combined differences obtained from the two columns is less than one and one-half times its standard error, so the difference is of doubtful significance. There is about 1 chance in 16 that the difference in the effect of the drying was accidental. The comparison presented in Table 2 gives no information on the magnitude of the difference between the effect of the drying on sporidia from *R. nigrum* and its effect on those from other *Ribes* species. The average of the germination percentages of sporidia from *R. nigrum* which received their preliminary dryings at saturation deficits between 0 and 6.9 mm. was 48 per cent; that of the comparable undried controls was 66 per cent. The preliminary drying reduced the average germination by approximately 18 per cent, on the basis of 100 per cent possible germination. On the basis of the germination actually occurring in the cultures it reduced germination about 27 per cent. The average of the germination percentages of sporidia from other species of *Ribes* which received their preliminary dryings at saturation deficits between 0 and 6.9 mm. was 35 per cent. That of the comparable undried controls was 59 per cent. The preliminary drying reduced germination about 24 per cent, on the basis of 100 per cent possible germination. On the basis of the germination actually occurring in the cultures it reduced germination about 41 per cent. The averages weighted by the number of sporidia counted and by the number germinating on each slide were approximately the same as those obtained by merely averaging the germination percentages of each slide.

Since a sporidium which does not germinate can not infect a white pine needle, even if all other conditions are favorable, the fact that even a brief drying reduces germination means that it reduces the possible number of infections from sporidia.

EFFECT OF ALTERNATE WETTING AND DRYING ON THE VIABILITY OF SPORIDIA

The effect of alternate wetting and drying of sporidia on their viability and their power to infect pines is another question of considerable practical importance, because in nature the sporidia are subjected to such conditions when dew or showers alternate with sunshine. Four groups of experiments yielding data on this problem were conducted with sporidia from different collections of *Ribes nigrum*. These included 14 series of tests, in 11 of which the films of water germinating the sporidia were obtained by laying the slides on ice for 1 or 2 minutes. In the first group of experiments some of the slides were dried and then wet once, some twice, and some three times. In the second and third groups the test slides received 1 to 5, and in the fourth group 1 to 11, alternate wettings and dryings.

Figure 5 gives a comparison of the germination of sporidia on iced slides which were dried 1 to 5 times at saturation deficits of 5 to 9 mm., with the germination of sporidia on iced slides dried at saturation deficits below 4 mm. The former were injured more by the drying than by the latter, beginning with the first drying and continuing to the fifth. The saturation deficits above 5 mm. decreased germination from the beginning, while those below 4 mm. did not begin to decrease germination until the third drying. In other words, the influence of the saturation deficits above 5 mm. was more marked than that of those below 4 mm.

Figure 6 gives a comparison of the comparable iced and uniced sporidia which were dried at saturation deficits of less than 4 mm.

The trend of both graphs is downward, but the one for the iced sporidia does not drop as low as does that for the uniced ones until the eighth drying. Beginning with the sixth drying, there is but one experiment for each of the following points. Hence the data for these should receive less weight than those based upon more experiments.

Even 11 alternate wettings and dryings did not kill all of the sporidia. This fact is emphasized more by the length of the germ tubes produced than by the percentages of germination. The maximum lengths of germ tubes produced in the two series in which there were 1 to 11 alternate wettings and dryings are shown in table on page 414.

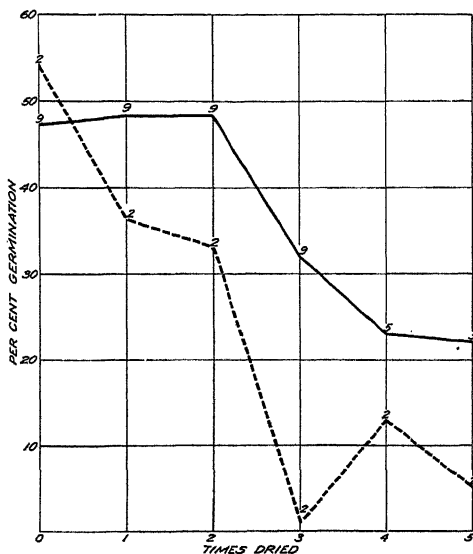


FIG. 5.—Effect of one to five alternate wettings and dryings on the germination of sporidia of *Cronartium ribicola*. All sporidia were wet by laying the slides on ice for 1 or 2 minutes. The figure at each point is the number of tests on which the point was based.

Solid line: Germination of sporidia dried at saturation deficits of 2 to 4 mm.
Dash line: Germination of sporidia dried at saturation deficits of 5 to 9 mm.

When these same slides were examined for evidences of pregermination it was found that pregermination was absent or slight, and that the tubes ranged from approximately 5 to 15 microns in length.

Number of times dried	Maximum lengths of germ tubes produced by—	
	Iced sporidia	Uniced sporidia
	<i>Microns</i>	<i>Microns</i>
0.....	40	60
1.....	50	40
2.....	50	50
3.....	50	20
4.....	25	30
5.....	30	50
6.....	30	30
7.....	60	
8.....	20	50
9.....	20	30
10.....	20	40
11.....	30	20

It is clearly shown by the above results that while a single brief period of dryness is harmful, as is shown by a comparison of the results obtained with the undried and the sporidia dried once (page 409), repeated drying is not as deadly as one would expect. In fact, a few sporidia showed remarkable resistance and germinated strongly, even after 11 dryings. Alternate wetting and drying was, on the

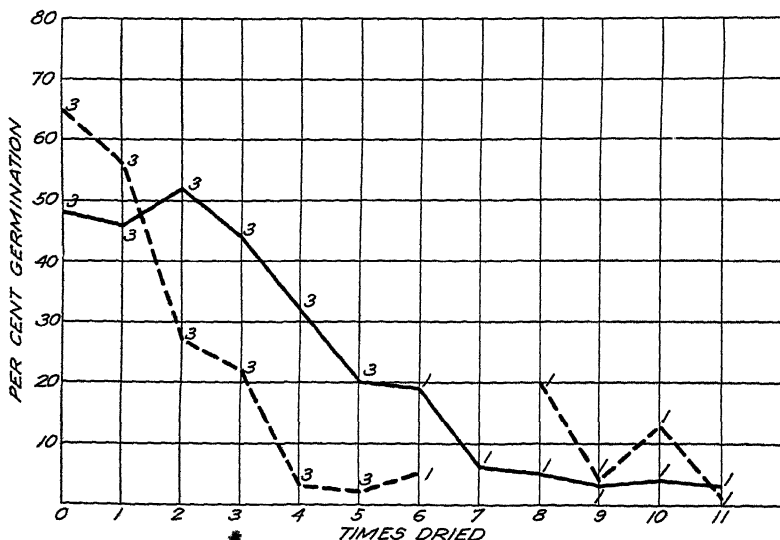


FIG. 6.—Comparison of the germination of iced and uniced sporidia of *Cronartium ribicola* alternately wet and dried 1 to 11 times at saturation deficits of 2 to 4 mm. The figure at each point is the number of tests on which the point was based.

Solid line: Germination of iced sporidia.
Dash line: Germination of uniced sporidia.

whole, less injurious when the sporidia were wet by icing than when they were wet by spraying. Drying of the iced sporidia was somewhat quicker than that of the uniced ones, as the film of water was thinner. It may be possible that if the sporidia had remained dry longer between wettings more injury would have been noted.

EFFECT OF PROLONGED DRYING UNDER UNCONTROLLED CONDITIONS ON THE VIABILITY OF SPORIDIA

To get an idea of the possible longevity of sporidia under the conditions existing out of doors during the season in which infection of pines occurs, slides of sporidia which had received the preliminary dryings were exposed to uncontrolled atmospheric conditions. These conditions were liable to sudden and decided change, as the weather at the season and place where the work was done was capricious. The relative humidity and temperature varied more or less while the experiments were in progress. At the beginning and at the end of each test the relative humidity and the temperature were recorded. From these the saturation deficits were calculated and the data were grouped into saturation-deficit classes. Where the conditions varied between two classes, the data was placed in the greater saturation-deficit class. Figures 7, 8, and 9 show the percentages of germination for the controls which had received only the preliminary dryings, and those for the sporidia which were further dried during exposure. They show that exposure of sporidia at all saturation deficits had an unfavorable effect on viability, but that some sporidia survived at all the saturation deficits in the tests.

In Figure 10 is a graph for each of the saturation-deficit classes, based on the difference between the average germination on the dried control slides and that on the exposed slides. Because of fragmentary data for the larger saturation deficits, the isolated points were connected with broken lines to show the general trend of each graph. Up to 12 hours' exposure, the graphs for the three classes run as would be expected—i. e., in the class of the smallest saturation deficit there was less difference between the dried controls and the exposed sporidia than there was in the other two classes. The graph for the medium saturation-deficit class occupies the middle position, and that for the class of greatest saturation deficit gives the greatest differences. This general trend is apparent throughout the graphs, in spite of the erratic fluctuations occurring at the longer times of exposure, these fluctuations probably being due largely to the scantiness of data. In general, within each saturation-deficit class the difference between the average germination of the exposed sporidia and that of the dried controls increased with the length of the exposure.

Table 3 gives data of the tests on the longevity of sporidia under natural (uncontrolled) conditions for the various species of *Ribes* from which the telia were taken. Three saturation-deficit classes, representing temperatures ranging from 32–86° F. and relative humidities ranging from 58 to 100 per cent, are used to show the conditions under which the sporidia were exposed. The upper limit of the third class was not the same for all species. Because the conditions were not controlled, the distribution of these tests in the three classes is purely accidental. The third column in Table 3 shows the tests in which germination occurred after the length of exposure indicated. The amount of germination is not given. The last column gives similar data for those tests in which no germination occurred after exposure. Figures 7, 8, and 9 show the amount of germination which occurred in these tests. Attention is called to the fact that there were relatively few tests in which germination entirely failed, even under severe conditions. It is believed that the failure to

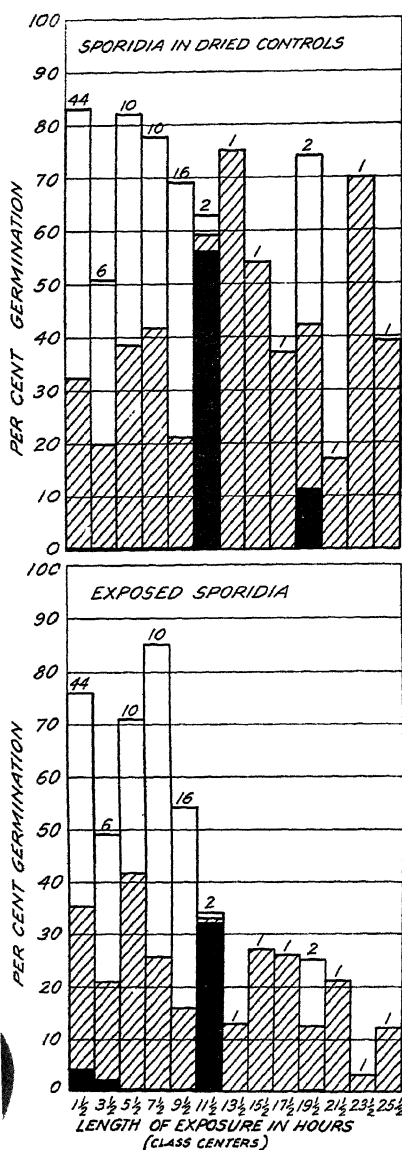


FIG. 7.—Germination of sporidia of *Cronartium ribicola* exposed to saturation deficits between 0 and 2.9 mm. The control slides had received the same preliminary drying and were comparable with the exposed ones, except for the exposure. The figure above each bar is the number of tests

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

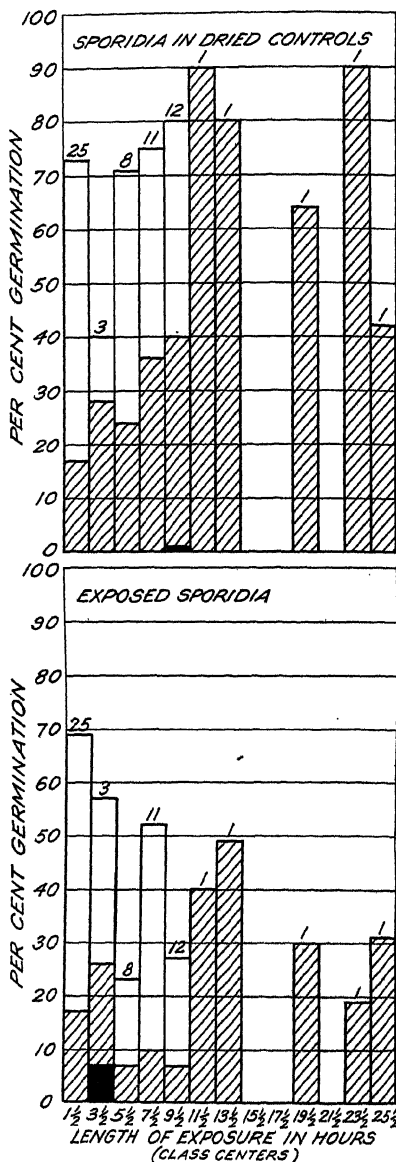


FIG. 8.—Germination of sporidia of *Cronartium ribicola* exposed to saturation deficits between 3 and 4.9 mm. The control slides had received the same preliminary drying and were comparable with the exposed ones, except for the exposure. The figure above each bar is the number of tests

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

germinate in certain of the tests is not due to the saturation deficits of the exposures but to undetected causes. Germination occurred in some of the tests at all periods of exposure up to 26 hours in length. No attempt was made to expose slides long enough to kill all of the sporidia, because the knowledge that they can remain viable in an air-dried condition for 26 hours is sufficient for all practical purposes. The vigor of germination of the sporidia exposed longest is indicated not only by the percentage of germinating sporidia but also by the maximum lengths of germ tubes formed in the cultures. The maximum tube lengths for the longest exposed sporidia from *Ribes rotundifolium* and *R. triste* were approximately 20 μ , only about 10 μ less than the maximum of those produced by the undried sporidia in the same series; and those for *R. odoratum* and *R. vulgare* were approximately 30 μ . No undried sporidia were tested in the same series. The maximum tube lengths for the longest exposed sporidia from *R. cynosbati* and *R. glandulosum* were approximately 40 μ , which equaled the maximum for the undried sporidia in the same series. The maximum tube lengths for sporidia from *R. nigrum* exposed 26 hours was 30 μ , which equaled the maximum for the unexposed sporidia in the same series. These figures seem to indicate that the prolonged drying did not reduce the vigor of the surviving sporidia as much as it did the percentage of germination.

Although the point has not been investigated for dried sporidia, it has been assumed that a sporidium which is capable of germinating is also capable of infecting white pines, if other conditions are suitable for infection to take place. In order to estimate the possible maximum number of viable sporidia per unit of leaf surface, the maximum germination percentages obtained after the longest exposures for

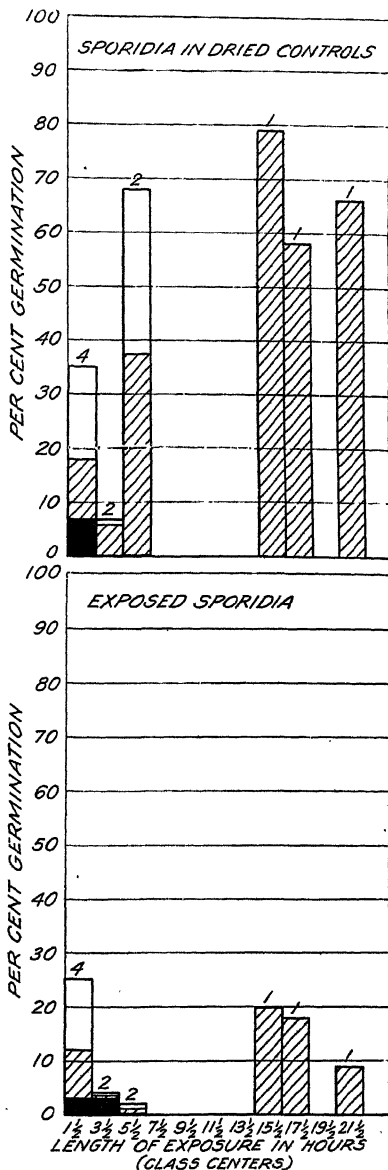


Fig. 9.—Germination of sporidia of *Cronartium ribicola* exposed to saturation deficits between 5 and 9.5 mm. The control slides had received the same preliminary drying and were comparable with the exposed ones, except for the exposure. The figure above each bar is the number of tests

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

each *Ribes* species were applied to the potential maximum sporidial production estimates given by Taylor (56). These give the astonishing figures in the fifth column of Table 4. Each of these sporidia would be potentially capable of causing infection of white pines, but as a matter of fact only a very small fraction of them would ever reach pine needles and very few of those that reach pine needles ever cause infection. Observational data show that this is the case. Further longevity tests are needed to get an average figure for the third column of Table 4. The writers in a previous paper (53) have discussed some of the conditions which would presumably result in white pine infections.

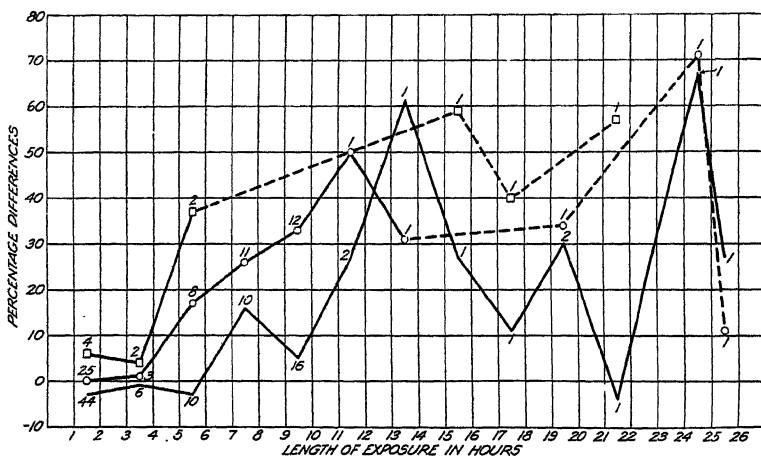


FIG. 10.—Differences between the average germination percentages of the exposed sporidia of *Cronartium ribicola* and those of the controls which had received only the preliminary drying. The figure at each point is the number of experiments upon which the point is based.

Solid line: Exposures to saturation deficits between 0 and 2.9 mm.

Circle, solid line: Exposures to saturation deficits between 3 and 4.9 mm.

Square, solid line: Exposures to saturation deficits between 5 and 9.5 mm

The broken sections of the lines indicate that the data were fragmentary.

TABLE 3.—*Longevity of sporidia of Cronartium ribicola from various Ribes hosts*

Ribes species	Saturation deficits (mm.)	Hour of exposure followed by germination	Hours of exposure followed by failure to germinate
R. americanum	0-2.9	1½; 1; 2; 3; 4; 5; 9½	
R. cynosbati	0-2.9	5; 6; 7; 7; 8; 8; 9; 9; 10; 10	9
	3-4.9	2	6
	5.0-5.7	1	
R. glandulosum	0-2.9	1; 1½; 2; 3; 4; 4; 5; 6; 7; 8; 9; 10	
	3-4.9	1½; ½; ½; 1; 1; 1½; 2; 2	
	5.0-9.5	3; 4; 6	5
R. nigrum	0-2.9	1½; ½; 1½; ½; 1½; 1½; 1½; 1½; ½; ½; 1; 1; 1½; 1½; 1½; 1½; 1½; 2; 2; 4; 5; 6; 6; 7; 8; 8; 9; 10; 10; 12; 14; 16; 18; 20; 22; 24; 26	5; 7; 9; 20½
	3-4.9	1½; ½; 1½; 1½; 1½; 1½; 1½; 1½; ½; 1; 1½; 2; 6; 8; 8; 9; 9; 10; 12; 14; 20; 24; 26	1; 1; 8; 10
	5.0-5.7	1½; 16; 18; 22	
R. odoratum	0-2.9	1½; 1½; ½; ½; ½; ½; ½; 1; 1; 1½; 1½; 2; 2; 10	
	3-4.9	6; 7; 8; 9; 10	
R. rotundifolium	0-2.9	½; 1; 1½; 1½; 2; 6; 8½; 9; 11½	
	3-4.9	½; 2; 3; 4; 5; 5; 6; 6; 7; 7; 9; 10; 10	8; 8
	5.0-6.2	1½; 2	
R. triste	3-4.9	8; 9	6; 7; 10
R. vulgare	0-2.9	9	

TABLE 4.—Potential number of viable sporidia of *Cronartium ribicola* per square inch of leaf surface after prolonged exposures while dry

Ribes species	Hours of exposure	Maximum percentage of sporidia germinating after the exposures given in the second column	Potential production of sporidia per square inch ^a	Number of potentially viable sporidia per square inch after the exposures given in the second column
<i>R. triste</i>	9	12	360,000	43,200
<i>R. americanum</i>	9½	54	974,280	526,111
<i>R. cynosbatl</i>	10	31	1,141,920	353,985
<i>R. vulgare</i>	9	11	1,305,000	143,550
<i>R. glandulosum</i>	10	22	1,679,484	369,486
<i>R. rotundifolium</i>	11½	32	2,746,140	878,765
<i>R. odoratum</i>	10	11	5,480,640	602,870
<i>R. nigrum</i>	14	49	16,799,400	8,231,706
<i>R. nigrum</i>	26	31	16,799,400	5,207,814

^a From publication by Taylor (56).

EFFECT OF PROLONGED DRYING UNDER CONTROLLED CONDITIONS ON VIABILITY OF SPORIDIA

As a phase of the investigations of the effect of the relative humidity and temperature of the surrounding air upon sporidia exposed dry, experiments were performed in which the relative humidity was controlled. This was done by means of sulphuric acid, a method long known and used by chemists for removing the water vapor from air contained in small chambers. So far as known to the writers, this method was applied only rather recently to controlling relative humidity of air in which fungus cultures were grown (30, 55, 57, 65). Scheibler desiccators of approximately 5,000 c. c. total capacity were used. Four hundred c. c. of sulphuric acid of known density was put in the base of each desiccator. This amount of acid was used because it presented a maximum surface area for the most rapid action on the air, and with such a volume the density of the acid would be changed very little by the water taken up from the air. Acid known in the trade as C. P., and of a specific gravity of 1.84, was used in making up the solutions. The acid was diluted with crushed ice in a flask embedded in ice and water. Density was ascertained with a hydrometer. Curtis (20) gives a graph showing the relative humidity which each density of sulphuric acid maintained in an inclosure. This graph appeared to be the most complete source for such data, his data agreeing within experimental error with data from other sources (55), and it was used in preparing the acid solutions for these experiments. As soon as the acid solution was put in the chamber, the cover was sealed with vaseline and the chamber was placed in the above-mentioned cellar, where the temperature was quite uniform. The acid was put in the chambers at least 12 hours before the beginning of a test, so that the relative humidity of the air could reach equilibrium. Tests were run simultaneously in five chambers, each at a different relative humidity. The covers were fitted with ground glass stoppers, the openings for which were large enough for an ordinary glass microscope slide to pass through them easily. A slide bearing sporidia could be inserted or taken out through these openings in about 10 seconds. This

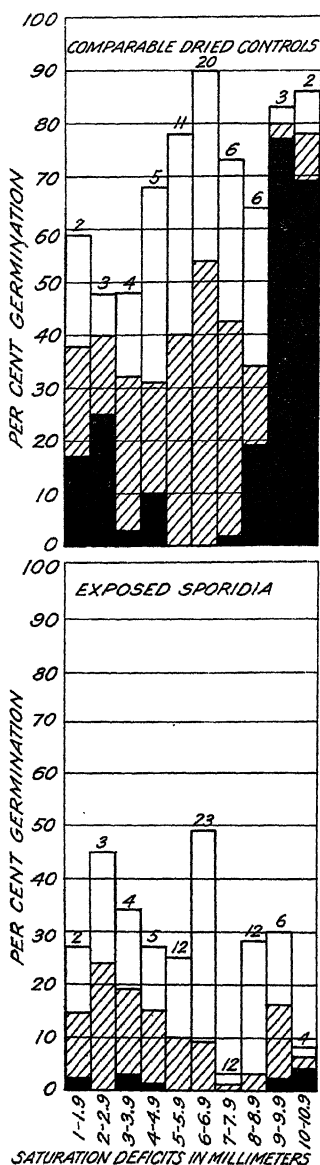


FIG. 11.—The germination of sporidia of *Cronartium ribicola* in the desiccators $3\frac{1}{2}$ hours. The control slides received only the preliminary drying. The figure above each bar is the number of tests. In a few cases there were more exposed slides than controls, because a single control slide sometimes served for two exposed

of. The black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

brief period allowed but little interchange of air, especially as the air surrounding the chambers was of the same temperature as that within them. Liberal time was allowed for the experiments, so that the relative humidity of the air could become stabilized after the opening of the chamber. A few experiments ran for $1\frac{1}{2}$ hours, and a few others for $5\frac{1}{2}$ hours, but most of them ran $3\frac{1}{2}$ hours. In the chambers, the slides of sporidia rested in a horizontal position upon a loose glass plate supported by a perforated porcelain plate. The slides were about half way, vertically, between the cover of the chamber and the surface of the acid. After a series of experiments was completed, the density of the acid was again determined. The temperature in the chambers was read without opening them, directly from thermometers lying on the glass plates beside the slides. Fluctuations of the temperature outside the chambers were so slight that there was practically no variation in the chambers during an experiment.

Eleven experiments were run at various times, with different material, and with saturation deficits ranging from 1.0 to 10.9 mm. which represented temperatures ranging from 53° to 70° F. and relative humidities ranging from 41 to 86 per cent. Most of the sporidia were wet by spraying, but a few were wet with the condensation water resulting from placing the slides on ice. Figure 11 shows (much condensed) the results of all of the $3\frac{1}{2}$ -hour tests. Since all factors, except that of saturation deficit at which they were exposed, are constant throughout each test, no error results from combining the results of both iced and uniced slides in the same graph. Figure 11 shows clearly that the material used for the tests at the higher saturation deficits happened to be most vigorous originally. This was purely accidental. Figure 12 represents the ratio of the average germination of the exposed sporidia to that of their dried controls. It shows that the smaller saturation deficits gave the higher per-

centages of germination, and that there was a decrease of germination as the saturation deficit increased. Figure 13 shows the differences obtained by subtracting the average percentages of germination of the exposed sporidia from those of their dried controls. The two methods of comparison give results that are in essential agreement with each other. Both show clearly that the injury to the sporidia from exposure increased with the increase of saturation deficit at which they were exposed.

Figure 14 shows the data for all comparable tests run at the three periods of exposure— $1\frac{1}{2}$, $3\frac{1}{2}$ and $5\frac{1}{2}$ hours—grouped according to the saturation deficits at which they were run, viz, 2.0 to 2.9 mm.

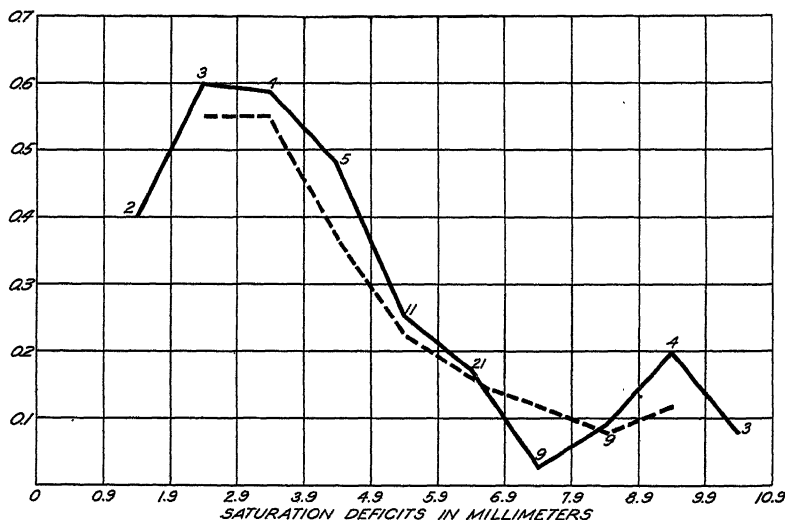


FIG. 12.—The ratio of the average germination percentages of sporidia of *Cronartium ribicola* in desiccators, $3\frac{1}{2}$ hours to the average germination percentages in the comparable controls. The figure at each point represents the weight given that point in making the 3-point moving average. This weight was obtained by averaging the number of control slides and the number of exposed slides, halves being dropped in all cases.

Solid line: Ratio between the germination percentages in exposed and the control slides.

Dash line: Weighted 3-point moving average of the same data.

3.0 to 4.9 mm., and 5.0 to 6.9 mm. The available data are limited, but they show quite clearly a general trend of higher germination at the smaller saturation deficits, and an earlier falling off of germination at the greatest saturation deficit. Also, increase in length of time of exposure tends to decrease germination. The graph for the saturation deficit class 3.0 to 4.9 mm. is abnormally irregular, but even with it the tendency is downward with increase in time of exposure.

EFFECT OF SUNSHINE ON VIABILITY OF SPORIDIA

In 1923 nine series of preliminary tests on the effect of direct sunshine on the viability of sporidia were run. In these tests a recording hygrothermograph was set in the full sunshine. The sporidia were exposed upon the black, perforated, metal plate, located just over the

hairs of the instrument and scarcely an inch from them. The material used was of low viability, as was all of that used in 1923. However, the controls, which had had the usual preliminary drying, gave consistently higher germination than did the sporidia which had been exposed to the sun for 2 to 12 minutes. Low percentages of germination were obtained after such exposure with the saturation deficit ranging from 1.0 to 18.0 mm. Sporidia which germinated after exposure to sun at a saturation deficit of 8.5 mm. produced germ tubes $20\ \mu$ in length, and secondary sporidia. No undried sporidia

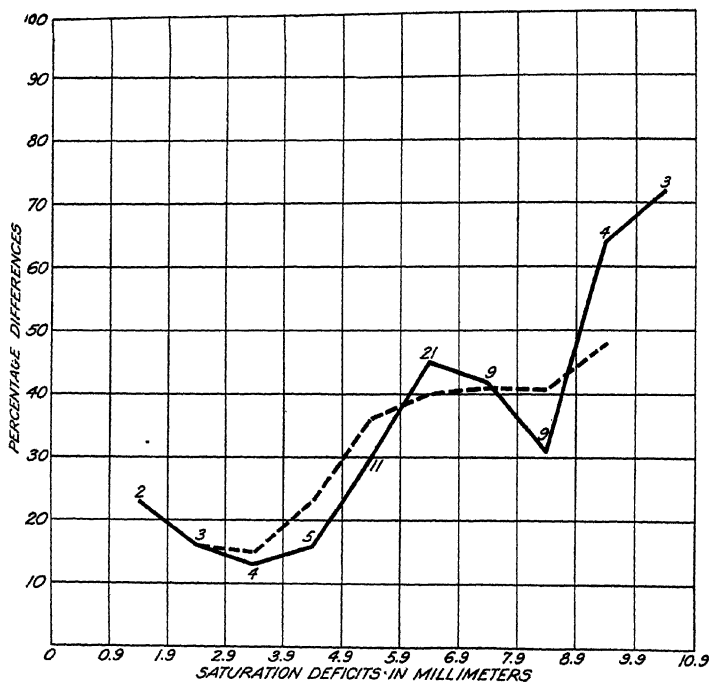


FIG. 13.—The differences between the average germination percentages of sporidia of *Cronartium ribicola* in desiccators $3\frac{1}{2}$ hours and those of the controls which had received only the preliminary drying. The figure at each point is the weight given that point in making the 3-point moving average. This weight was obtained by averaging the number of control slides and the number of exposed slides, halves being dropped in all cases.

Solid line: The differences between the germination percentages in the exposed and the control slides.
Dash line: Weighted 3-point moving average of the same data.

were tested in these series, so it is impossible to state how long the germ tubes would have been which these sporidia would have produced prior to their preliminary drying and exposure.

In 1924 an attempt was made to eliminate the heat of sunlight as an injurious factor. In the first attempt it was expected that the heat from the sun would be great enough to prevent water vapor from condensing on the slides. The sporidia were exposed to sunlight within Petri dishes lined below with moist filter paper, and set upon a flat surface. A film of water condensed immediately on the upper surfaces of the slides, thus wetting the sporidia. For the six series of tests thus made, the average of the percentages of germination in the

dried controls and that of the sporidia exposed as above described was practically equal, being 31 and 32 per cent respectively. That is, the water film, the glass cover, or the low temperature, prevented any injury from the exposure to the sun. Sporidia exposed at the same time by placing the slides on a light-colored surface in direct sunlight were decidedly injured. This may mean that the heat rays rather than the visible rays of sunlight injure sporidia, or that ultra-violet rays or some other rays which glass cuts out injure them. In a further attempt to eliminate the heat, slides were placed within dry Petri dishes seated on a flat ice surface. A film of water condensed even then. No sporidial tests were made by this method. Finally, one-half of a Petri dish was turned bottom side up on a flat cake of ice. Slides were then placed for exposure upon the glass surface, which was cooled by the imprisoned cool air held between the dish and the ice. No water film could be detected under these conditions,

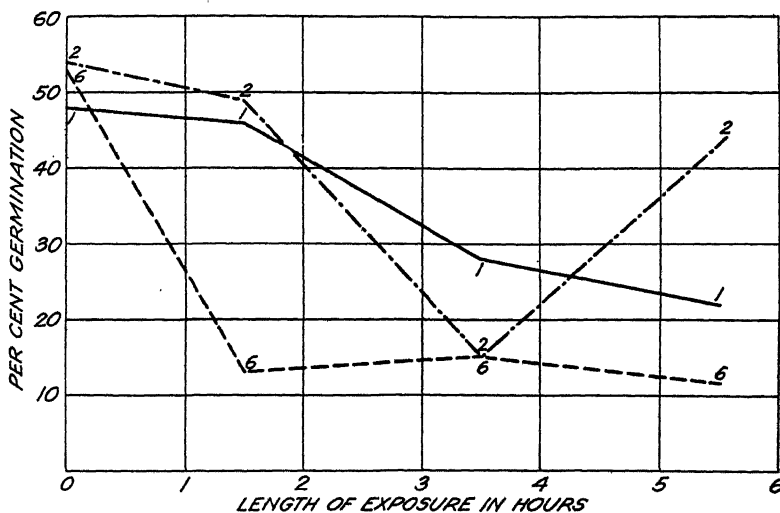


FIG. 14.—Comparison of the effect of exposure of sporidia of *Cronartium ribicola* for different periods at different saturation deficits. The figure at each point is the number of tests on which the point is based

Solid line: Saturation deficits between 2 and 2.9 mm.

Dot-dash line: Saturation deficits between 3 and 4.9 mm.

Dash line: Saturation deficits between 5 and 6.9 mm.

although the slides remained cool to the touch. Most of the heat was apparently eliminated without water condensing on the glass. In all of these tests, in order to eliminate the cooling from the ice as an additional factor influencing the germination of the exposed sporidia, some of those exposed without icing were, after exposure, iced for an equal length of time. Figure 15 shows the results for all tests, regardless of the length of exposure. All were low in germination, so the length of exposure within the periods in these experiments does not seem to be significant. The graph for the undried controls shows consistent higher germination at a fairly constant level than that which occurred in either the dried controls or the exposed slides. The dried controls show a rather high germination as compared with the exposed slides, but decidedly lower than the undried controls. The exposed sporidia gave less than 5 per cent germination, but continued to give this low percentage of germination at all saturation

deficits encountered in this series of experiments (5.0 to 22.5 mm.). The exposure to the sunlight was practically equally injurious through the entire series of experiments, regardless of the length of exposure or the saturation deficit at which they were exposed.

Figure 16 shows the results of certain experiments where exposure to sunlight was made for 30 minutes. The sunlight killed nearly all of the exposed sporidia, but not all, even at the large saturation deficit of 23 to 24 mm. Here again the preliminary drying as shown by the dried controls had a decidedly unfavorable effect on viability, as compared with the undried controls.

Figure 17 shows a comparison of the averages of the various kinds of sunshine exposures in three different experiments. In all three

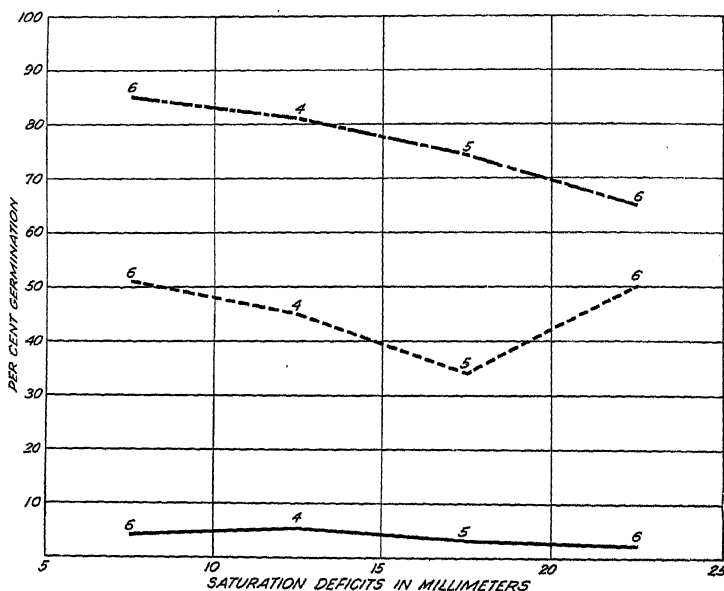


FIG. 15.—Effect of exposure of sporidia of *Cronartium ribicola* to direct sunlight at different saturation deficits. The figure above each point is the number of tests on which the point was based

Solid line: Germination on slides exposed to sunlight.

Dash line: Germination on comparable control slides which had received only the preliminary drying.

Dot-dash line: Germination on comparable control slides which had received neither the preliminary drying nor the subsequent exposure.

experiments the material was of approximately equal viability, as is clearly shown by the germination of the undried controls. These graphs show (1) that the preliminary drying decreased viability decidedly; (2) that exposure in the sunlight (on ice to eliminate heat) injured them still more; (3) that exposure in sunlight to full effect of light and heat caused still greater injury; and (4) that icing after exposure to sunlight, as in 3, had no perceptible stimulating effect; that is, the injury from the sunlight was so great that icing after exposure did not stimulate germination.

Finally, attention should be called to the remarkable persistence of viability in a few of the sporidia which were exposed to the rigorous conditions of complete and unaltered sunlight. That germination really occurred on these slides after they were exposed there can be

no doubt. For instance, at a saturation deficit of 9.0 mm. germ tubes of a maximum length of $50\ \mu$ were produced. Sporidia exposed at a saturation deficit of 15 mm. produced germ tubes with a maximum length of $30\ \mu$, and also secondary sporidia. Those exposed at a saturation deficit of 19 mm. produced germ tubes up to $40\ \mu$ long. And after exposure at a saturation deficit of 24 mm. germ tubes $30\ \mu$ long were formed. The germ tubes of maximum length in the undried controls were of approximately the same length as these, or $20\ \mu$ shorter.

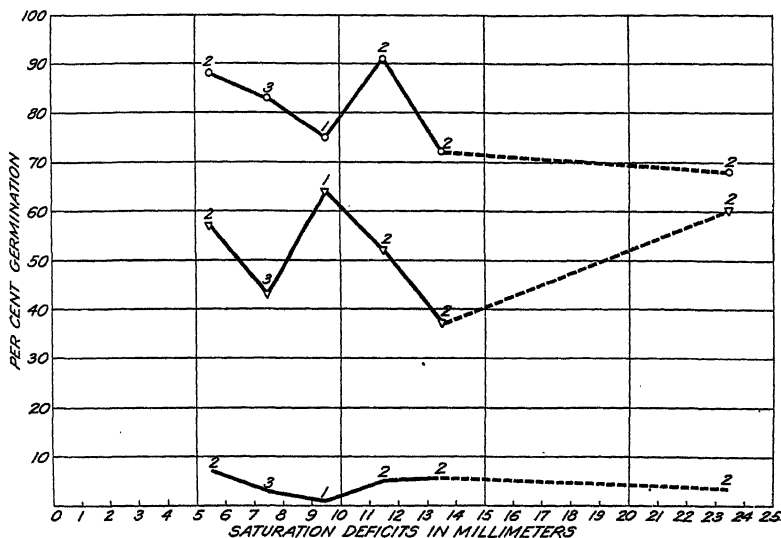


FIG. 16.—Effect of exposure of sporidia of *Cronartium ribicola* to sunlight for 30 minutes at various saturation deficits. The figure above each point is the number of tests on which the point was based. The broken sections of the lines indicate that the data were fragmentary

Solid line: Exposed to sunlight.

Circle solid line: Comparable undried controls.

Triangle solid line: Comparable dried controls.

EFFECT OF ICING ON VIABILITY OF SPORIDIA

During this study much difficulty was experienced in wetting dried and exposed slides with a film of water which was thin enough to give the best germination of the moistened sporidia. Spraying with an atomizer did not seem to give a continuous sheet of water which was thin enough for the best results under all conditions. Finally, upon the suggestion of L. H. Pennington, condensing moisture on the slides by putting them on ice was tried. The film of condensed moisture could be made heavier by prolonging the period of icing, but a perceptible continuous film could be obtained in one minute. Since a thin but continuous film of water was very desirable, this method of wetting the slides of dried sporidia was used in many of the experiments, in spite of the obvious undesirability of introducing an additional variable factor. The use of this method led to the incidental accumulation of data on the effect of icing upon the viability of the sporidia. In addition to this, some definite experiments were run under different conditions to determine whether icing really has an invigorating effect on the sporidia after they have

been weakened by adverse conditions. Ericksson (25, 26) said that precooling seemed to stimulate and increase the germination of rust spores, but the present writers (54), in some preliminary tests of the effect of cooling upon the germination of the teliospores of *Cronartium ribicola*, found no evidence that precooling per se increased their germination.

For general comparison of the iced and uniced sporidia the results of eight groups of experiments were available. There were 54 pairs of slides in which the two were given identical treatments, except that one was iced just before germination, while the other was not. Twenty-six of the 54 iced slides produced secondary sporidia, whereas only 18 of the 54 uniced ones did so. The mean germination for the 54 iced slides was 27.2 per cent, and for the 54 uniced slides it was 17.6. This method of averaging gives excessive weight to the pairs of slides with better material.

Another method of comparing the effect of cooling and noncooling which gives equal weight to all the individual comparisons is to consider the proportion of the comparisons in which the germination on the iced slides was better than that on the uniced ones. Of the 54 comparable pairs of slides the iced slide showed better germination in 35 pairs, and the uniced one in 17, while in 2 pairs none of the sporidia germinated on either slide. These last two pairs may be considered simply as tests in which a decision was prevented. In the 52 cases in which there was a difference between the iced and the

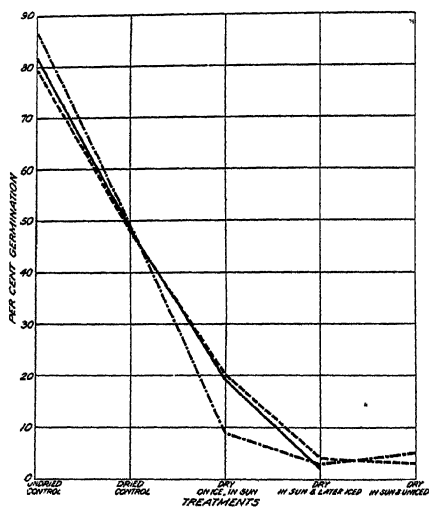


FIG. 17.—Comparison of the germination which followed different treatments of sporidia of *Cronartium ribicola* in three different experiments

Dot dash line: First experiment.
Solid line: Second experiment.
Dash line: Third experiment.

uniced slides the most probable result, if the icing had had no effect, would have been superiority of the iced slides in half of the comparisons and of the uniced ones in the other half. In other words, the iced slides should have been expected to be superior in only 26 of the cases. The standard error of this expectation, proceeding on the assumption that p (the probability of the iced slide being best) equals q (the probability of the uniced one being best), and that each equals $\frac{1}{2}$, is found by the formula $(p \times q \times n)^{\frac{1}{2}}$ (32), which, in this case, is $(\frac{1}{2} \times \frac{1}{2} \times 52)^{\frac{1}{2}}$, or 3.6. The observed results that the iced slide was better in 35 of the cases differs from the expectation by 9, two and one-half times the standard error. If p and q are really equal as assumed, the odds would have been about 160 to 1 against the iced slide being superior to the uniced slide in as many as 35 cases. The odds, therefore, on the face of the results, are 160 to 1 that the assumption that icing is ineffective is an incorrect one. In other

words there is a rather high probability that icing did increase the amount of germination. The fact that the uniced slide was superior in so many cases would, on this basis, be taken as evidence that the effect of icing, while real, was less in magnitude than the variations due to fluctuations in sampling the sporidia or to some of the unknown and uncontrolled factors which made for variability.

Conclusions based on the above probability computations must be accepted with some reservations. Aside from the assumptions which must always be made in any application to biological data of methods based on the normal frequency curve, a further difficulty enters in, in that the population of comparisons was not a simple population. Some of the pairs had been exposed to drying or sunlight for a long time, some for a short time, and some not at all. Some were made at one time and some at another, some with material from one source and some material from another. It is thus entirely possible that some slides were affected differently from others; that in some tests the icing was beneficial, but that in slides which had received other types of preliminary treatment the icing was without effect or was even harmful. In any case, however, it is difficult to avoid the conclusion that the icing caused more germination on some slides.

To get information on whether icing affected both poor-germinating and well-germinating sporidia, the germination in each iced slide was averaged with its comparable uniced one. Then the population was divided into two groups, one group including the 27 pairs with the lowest average germination; and the other the 27 pairs with the highest average germination. The former group contained 17 pairs in which the iced slides germinated better than the uniced ones, 8 in which it germinated more poorly, and 2 in which there was no germination in either iced or uniced. In the latter group there were 18 pairs in which the iced slide germinated better, and 9 in which the uniced one germinated better. This indicates that despite the absence of true simple sampling, the population of the 52 pairs of slides was quite homogeneous, as far as reaction to icing is concerned.

Three groups of experiments were run to determine the effect of different periods of icing—ranging from 1 to 50 minutes in length—upon the germination of sporidia. These data were compared by these two methods: direct comparisons of the percentages of germination, and ranking by decrease in germination. The results in both methods are inconclusive. So far as these tests show, after 1 or 2 minutes there was no perceptible added effect from increasing the length of icing. In other words, a short period of icing (1 to 5 minutes) apparently has just as much stimulative effect as a longer one.

If icing stimulates germination of sporidia, as it sometimes seems to do, then sporidia like those which Spaulding (52) found being produced on snow would be stimulated to increased germination and they probably could cause infections late in the season. There is a possibility that the apparent effect from icing was not a temperature effect but a moisture effect, since a thinner and more even film of water could be deposited by icing than by spraying.

In working up the above data no attempt was made to separate the effect of temperature from that of drying. There is a possibility that the temperature at which the sporidia were dried may have had an effect independent of that of drying. This temperature relation might account in part for the failure of most sporidia exposed by York at North Conway to germinate (51). This phase

of the problem should receive further investigation. Dole (22), in studying the effect of air temperature and relative humidity on the transpiration of *Pinus strobus*, found that "the products of actual losses and vapor pressure may be correlated with temperature, which means that temperature has an additional influence on the phenomenon other than its influence in determining the value of vapor pressure. This additional influence may be interpreted in terms of diffusion." If there were a separate temperature effect on the sporidia, it would probably be of the nature of a biochemical change in the protoplasm.

PRODUCTION OF SECONDARY SPORIDIA

Secondary sporidia are rather commonly produced by the various genera of the Uredinales (2, 3, 4, 5, 6, 7, 8, 9, 16, 18, 28, 38, 40, 41, 43). They were known by early workers (43) for some species of *Cronartium*, but Colley (18) seems to have been the first to report them for *Cronartium ribicola* in the literature.

Secondary sporidia were common in the experiments reported here (fig. 2), and the writers regarded them as an indication of vigorous sporidial germination. They were formed in small numbers on some slides, but usually they occurred in large numbers if at all. In a few cases tertiary sporidia were formed (fig. 2).

Fresh teliospores, as well as those which had been stored for some time, produced sporidia which in turn produced secondary sporidia when they germinated. Teliospores from *Ribes rotundifolium* stored out of doors in mosquito-netting bags for 21 days; from *R. americanum* stored for 38 days; from *R. triste* stored for 47 days; from *R. vulgare* stored for 49 days; from *R. nigrum* stored for 52 days and from *R. glandulosum* stored for 55 days, were all able to germinate and produce sporidia which were vigorous enough to in turn produce secondary sporidia.

Twenty-six of the iced slides in the 54 comparable pairs of iced and uniced slides produced secondary sporidia, whereas only 18 of the uniced ones did so. This suggests, but does not prove, that icing may stimulate the production of secondary sporidia.

Since Reed and Crabill (40) found that the sporidia of *Gymnosporangium juniperi-virginianae* produced secondary sporidia only when they were kept continuously wet from the time of their formation until the time of their germination, an attempt was made to ascertain the effect of drying upon the formation of secondary sporidia by *Cronartium ribicola*. All the strictly comparable pairs of undried control slides and slides subjected to preliminary drying were examined for the production of secondary sporidia. In 64 such pairs only one slide produced secondary sporidia. In 43 of the 64 pairs, the undried control produced the secondary sporidia, while the dried controls of but 21 pairs produced them. The significance of the apparent superiority of the undried controls in ability to produce secondary sporidia will be examined by the method employed on page 426. Assuming that the drying had no effect, the most likely result would be that in 32 of the pairs it would be the undried controls which produced secondary sporidia and that in 32 of the pairs it would be the dried controls. The standard deviation of the expected number is $\sqrt{\frac{1}{2} \times \frac{1}{2} \times 64} = 4$. The difference between expected and observed results is $43 - 32$, or 11, which is $2\frac{3}{4}$ times its standard

error. Then the odds are 330 to 1 that the preliminary drying really did decrease the ability of the sporidia to form secondary sporidia.

Besides these 64 pairs, there were 43 pairs in which neither slide produced secondary sporidia, and 75 pairs in which both produced them. These were cases in which the material was too poor or too good to give differential results by the rather crude criterion employed, so they probably may be disregarded. In other words, 65 per cent of the undried slides produced secondary sporidia, whereas only 53 per cent of the comparable slides which had received the preliminary drying did so.

Long exposure of sporidia in an air-dried condition did not prevent the formation of secondary sporidia; in fact, in 1923 the exposed sporidia produced secondary sporidia just as readily as those which received only the preliminary dryings. Some of the sporidia from *Ribes nigrum* which had been alternately wetted and dried 11 times produced secondary sporidia. For sporidia from each of the eight *Ribes* hosts the maximum exposure in an air-dried condition, after which secondary sporidia were produced, were as follows: *Ribes americanum* 5 hours; *R. cynosbati* 10 hours; *R. glandulosum* 10 hours; *R. nigrum* 26 hours; *R. odoratum* 1 hour; *R. rotundifolium* 3½ hours; *R. triste* none; and *R. vulgare* none. Few tests were made with the last two species, and it is probable that further tests would have shown that exposed sporidia from even these two could produce secondary sporidia. If, as Clinton and McCormick (16) suggests, "this formation of a secondary sporidium is probably to tide it over unfavorable conditions of infection," sporidia of *Cronartium ribicola*, which in turn produced secondary sporidia, are potentially dangerous to pines for much longer periods than the longevity of the primary sporidia would indicate.

The facts that teliospores which have been stored for a long period can produce sporidia which are vigorous enough to produce in turn secondary sporidia, and that sporidia exposed dry for some time can produce secondary sporidia, lengthen the time during which a given generation of sporidia is potentially capable of causing infection.

SUMMARY

The present investigation is the first attempt to make a detailed physiological study of the sporidia of any species of *Cronartium*. Such a study was undertaken with *C. ribicola*, because it is impossible to understand the nature and conditions of pine infection without a knowledge of the infecting sporidia.

The influence of physical factors, particularly drying, upon the sporidia from eight *Ribes* hosts, has been studied. Methods for making studies of this type have been developed.

The time necessary for the production of sporidia increased directly with the length of storage of the telial material. Temperature also influenced the time necessary for sporidial production. Sporidia appear to need water for germination, but the thinnest film is sufficient.

The viability of wet sporidia was decreased by merely bringing them to an air-dried condition and immediately rewetting them. The amount of injury increased directly with the increase in the saturation deficit of the air in which they were dried. This injury was shown in most cases by the reduction in the germination percentages rather than in vigor of growth.

A few sporidia were able to survive as many as 11 alternate dryings and wettings, but their ability to germinate tended to decrease with each successive drying. When the atmospheric saturation deficits at which the successive dryings occurred were less than 4 mm. the injury to the viability of the sporidia was less than when they were more than 5 mm.

In desiccators where the relative humidity of the air was controlled by sulphuric acid of known specific gravities, the viability of the sporidia decreased directly with increase in the saturation deficits of the air. No injury from acid fumes was detected.

Sporidia from *Ribes americanum*, *R. cynosbati*, *R. glandulosum*, *R. nigrum*, *R. odoratum*, *R. rotundifolium*, *R. triste*, and *R. vulgare* survived exposures of nine or more hours in an air-dried condition. Some air-dried sporidia from *R. nigrum* survived exposure for 26 hours (the longest period tested) in air with saturation deficits between 2.2 and 3.4 mm. The viability of the sporidia tended to decrease with increases in the length of exposure and with increases in the saturation deficits of the air in which exposure occurred.

The thin continuous film of water obtained by icing the slides was most favorable for the germination of the sporidia. Precooling (icing) appeared to somewhat stimulate the germination of sporidia. Iced sporidia were less injured by successive dryings than were uniced ones. The length of the period of icing (if not less than one minute) appeared to be immaterial.

Small percentages of the sporidia survived short exposures in full sunlight with saturation deficits up to 23.5 mm.

Sporidia from all the tested species of *Ribes* produced secondary sporidia. Sporidia which were kept constantly wet appeared to produce secondary sporidia somewhat more readily than did the sporidia which had been dried.

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BIOLOGY OF THE SAW-TOOTHED GRAIN BEETLE, *ORYZAEPHILUS SURINAMENSIS* LINNÉ¹

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INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* Linné, one of the best known of the insects that attack stored foods, is cosmopolitan in distribution and is likely to be found in almost any stored food of vegetable origin. Although it has been known to scientists for more than 150 years, the statement by Chittenden (8, pp. 16-17)² that "during the warmest summer months the life cycle requires but 24 days; in early spring, from 6 to 10 weeks" is practically the extent of the information that the present writers have been able to find in the literature on the biology of this important pest.

HISTORICAL

Naturalists probably were familiar with this beetle long before its description by Linné in 1767 (25). Redi (29, Table XVII), in 1671, mentioned and figured an insect which resembles *Oryzaephilus surinamensis*, and which Wheeler (36) considers to be this or a closely allied species. Linné received specimens of this insect from Surinam (Dutch Guiana) and for that reason gave it the specific name *surinamensis*. Comparatively little seems to have been written by the early scientists on the biology of this insect. Westwood (35, p. 153) reported in 1839 that he had discovered it in sugar, and stated in 1848 that he had observed both larvae and adults floating in tea or coffee sweetened with infested sugar.

In 1846 there appeared in the Cultivator (32) an account of the experiments of a Mr. Rich of Shoreham, Vt., against an insect that was infesting his mill. He found that fumigating with brimstone smoke and tobacco smoke had little effect, but that the liberal application of boiling water and whitewash destroyed the insects. According to Gavit (17, pp. 661-662) the insect was the saw-toothed grain beetle, and a short account of it by him, with illustrations of the different stages, was published in 1849.

The same year Blisson (5) gave a detailed description of the immature stages, with brief notes on the habits and transformations of the insect. In 1853 Perris gave a brief account of the habits of the larva (28, pp. 628-631). Washburn (33) conducted a large series of experiments with this insect, and in 1890 he reported his results, with recommendations for control. Chittenden, in 1896, gave brief notes on the habits of the beetle (8, pp. 16-17; 9, pp. 121-122). He found that the life cycle required from 6 to 10 weeks in early spring, and about 24 days in midsummer in the vicinity of Washington, D. C. Dean (10, pp. 202-204) obtained similar results in Kansas,

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² Reference is made by number (italic) to "Literature cited," p. 451.

stating that the pupal stage required from 6 to 12 days and that in Kansas there were from four to six generations annually.

SYNONYMY

Linné, whose description of the insect in 1767 seems to be the earliest in the literature, named it *Dermestes surinamensis* (25, p. 565). Its cosmopolitan distribution and varied food habits attracted the attention of many scientists, and in the next few years after Linné's account it was redescribed under several different names. Fabricius (14, p. 62), in 1775, described it as *Anobium frumentarium*. De Geer (18, p. 54) the same year placed it in another genus, referring to it as *Tenebrio surinamensis*. Olivier (26, no. 18, p. 10), in 1790, referred to it as *Ips frumentaria*; and Fabricius (15, pt. 2, p. 496), in 1792, placed it in still another genus, referring to it as *Colydium frumentarium* and giving as synonyms all of the aforementioned names. In the same year (1792) Fabricius described the same species under the names *Dermestes sexdentatus* (15, pt. 1, p. 232) and *Scarites cursor* (15, pt. 1, p. 96). Kugelann in 1794 (24, p. 566) referred to it as *Lyctus sexdentatus*, Paykull in 1800 as *Colydium sexdentatum* (27, p. 313), and Gyllenhal in 1813 (21, p. 406) as *Silvanus sexdentatus*. In 1830 Stephens (30, p. 104) followed Gyllenhal in placing the species in the genus *Silvanus* but used the specific name *surinamensis*, and from that time until very recently it has been known to economic entomologists as *Silvanus surinamensis*. Ganglbauer in 1899 (16, pp. 583-584) revised the old genus *Silvanus* and placed the species *surinamensis* in the new subgenus *Oryzaephilus*. This revision has been accepted by most entomologists, and the saw-toothed grain beetle is now known as *Oryzaephilus surinamensis*.

Oryzaephilus surinamensis Linné:

- Dermestes surinamensis* Linné, 1767, Syst. Nat. ed. 12, 1 (2): 565.
- Tenebrio surinamensis* De Geer, 1775, Mém. Ins., 5: 54, pl. 13, fig. 12.
- Anobium frumentarium* Fabricius, 1775, Syst. Ent. 1: 62.
- Ips frumentaria* Olivier, 1790, Ins. 2 (18): 10, pl. 2, fig. 13.
- Colydium frumentarium* Fabricius, 1792, Ent. Syst. 1 (2): 496.
- Dermestes sexdentatus* Fabricius, 1792, Ent. Syst. 1: 232.
- Scarites cursor* Fabricius, 1792, Ent. Syst. 1: 96.
- Lyctus sexdentatus* Kugelann, 1794, Schneid. Mag. 1: 566.
- Colydium sexdentatum* Paykull, 1800, Faun. Suec. 3: 313.
- Silvanus sexdentatus* Gyllenhal, 1813, Ins. Suec. 1 (3): 406.
- Silvanus surinamensis* Stephens, 1830, Ill. Brit. Ent. Mandibulata 3: 104.
- Oryzaephilus surinamensis* Ganglbauer, 1899, Käfer 3 (2): 584.

LOSSES

Losses caused by the saw-toothed grain beetle are often considerable. Dried fruit (fig. 1) stored for considerable periods before packing invariably becomes infested, and in addition to a direct loss in actual destruction of fruit an expense is incurred in eliminating the insect in the process of preparing the fruit for packing.

The damage done by the beetle to stored seeds is often considerable, although its attack usually follows that of other insects such as the rice weevil. Adults confined with only sound wheat invariably die from starvation. Yet the saw-toothed grain beetle, often called the "bran bug" by grain dealers, has sometimes proved to be the chief offender in grain shipments, particularly to grain held in and shipped from the Northwest.

The presence of the saw-toothed grain beetle in foodstuffs, particularly in cereal products such as flour, meal, and breakfast foods, causes loss by rendering the food unsalable or unpalatable. The beetle's flattish form allows it to penetrate apparently tightly wrapped

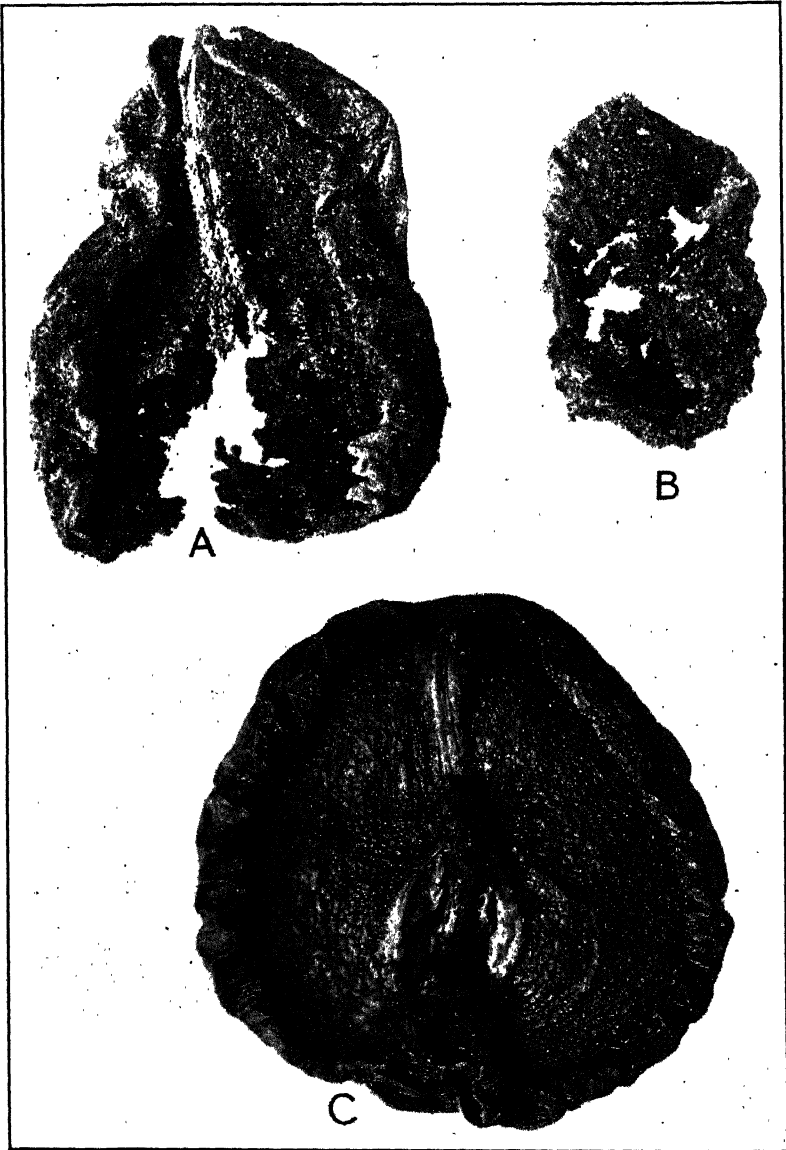


FIG. 1.—A and B, dried pears damaged by *Oryzaephilus surinamensis*; C, a normal dried pear

packages. The writers visited one grocery store that was overrun with this beetle and found that it had penetrated, with few exceptions, every box of crackers, breakfast cereals, and other packaged foods in the store. The presence of the beetles in so many com-

modities resulted in a rapid falling off of 50 per cent of the daily sales, and finally in a complete suspension of operations. The infestation in this case came from a barrel of scratch feed stored in a back room. The beetles multiplied so rapidly that they migrated to all parts of the store. This migratory habit was observed by Taschenberg (31, pp. 19-20) nearly 50 years ago. According to his account, the beetles became so numerous in a brewery that on warm

days they invaded the neighboring houses, even crawling into the beds and nipping the occupants at night. A correspondent reported in 1922 from Seattle, Wash., that adults migrated in hordes from a feed store adjoining the residence and so overran the pantry and living rooms that nothing but the coming of cold weather ended the difficulty.

BIOLOGY

In the vicinity of Washington, D. C., the insect passes the winter in the adult stage, breeding normally ceasing late in the fall and commencing again in the spring. In heated buildings, if the relative humidity is not too low, breeding will continue at a slow rate throughout the winter. Most heated buildings, however, are so dry that little or no breeding takes place, although the adults remain active throughout the winter.

Beetles kept during the winter in an incubator, in which the relative humidity was high, bred more or less freely. In tropical climates breeding undoubtedly continues throughout the year.

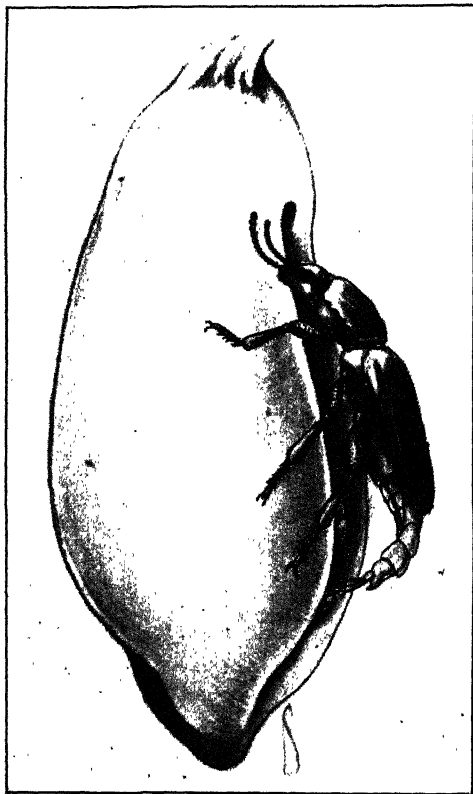


FIG. 2.—*Oryzaephilus surinamensis*: Female ovipositing on kernel of wheat. Note extended ovipositor and three eggs already deposited. All greatly enlarged

THE ADULT

The adult is a very active, slender, flattish, brown beetle, about one-tenth of an inch long (fig. 2). It has well developed wings, but rarely if ever flies. The posterior femora of the males, as Guillebeau (20, p. 220) has pointed out, are each armed with a tooth, which serves as an excellent character for distinguishing the males from the females, the femora of which are unarmed. The adult is nearly omnivorous, and many entomologists have published accounts of its

food habits. In this connection, Wheeler (36) very aptly states, "It is cosmopolitan and gregarious; living in nearly all stored human foods of vegetable origin; cereals (rice, wheat, maize, barley, etc.), ground or unground or in the form of paste (macaroni), bread etc.; dried fruits, nuts, copra; more rarely in sugar, starch, drugs, tobacco, snuff, or dried meats."

LONGEVITY

The beetle is rather long-lived. One specimen reared in the laboratory lived for 3 years and 3 months. This specimen was a male that transformed July 6, 1921. It was placed with a female for breeding records, but the female died shortly thereafter and the male was kept segregated for the rest of its life. Several other males reared in the laboratory lived for more than 2 years. Of the females reared in the laboratory, a majority of them lived for from 6 to 10 months, a few lived longer than 1 year, but only one lived for more than 2 years. This last-mentioned individual lived for 2 years and 8 months. All of these females were mated and they laid a considerable number of eggs. Data on the longevity of 10 females are given in Table 1.

TABLE 1.—Data on the oviposition and longevity of 10 females of *Oryzaephilus surinamensis*, at Washington, D. C.

No.	Date emerged	Date first egg was laid	Length of preoviposition period	Date last egg was laid	Length of oviposition period	Number of eggs laid	Date of death	Length of life
	1921	1922	Days	1922	Days			Days
1.....	Aug. 31	Mar. 26	207	June 26	92	226	July 1, 1922	304
2.....	Oct. 8	Apr. 2	176	Aug. 11	131	117	Aug. 14, 1922	310
3.....	Nov. 3	Apr. 1	149	June 17	77	167	Aug. 2, 1922	272
4.....	Nov. 7	do.	145	Aug. 24	145	239	Jan. 17, 1923	436
5.....	do.	Mar. 18	131	Aug. 7	142	103	Nov. 17, 1922	375
6.....	Dec. 21	Mar. 24	93	July 19	117	96	July 28, 1922	219
	1922							
7.....	Feb. 12	do.	40	Aug. 14	143	285	Dec. 15, 1923	671
8.....	Feb. 15	Mar. 22	35	do.	145	216	Oct. 8, 1924	966
9.....	Mar. 20	Apr. 2	13	July 18	107	45	Aug. 7, 1922	140
10.....	May 30	June 7	8	Aug. 28	82	235	Aug. 20, 1923	447

OVIPOSITION

The eggs are laid singly or in small clusters, and are usually tucked away out of sight in some crevice in the food supply selected or laid loosely in finely ground foods such as flour or meal. In obtaining egg-laying records in the laboratory, split kernels of corn were furnished as food and as repositories for eggs. When only one or two pieces of corn were supplied it was an easy matter to obtain the daily egg record.

Oviposition was repeatedly observed. In one typical instance the female maneuvered around a broken kernel of corn, feeling here and there with her ovipositor, apparently seeking a crevice in which to deposit her egg. After several minutes, during which she explored all of the kernel, she finally settled on a small crevice in the germ part, and into this crevice she thrust her ovipositor and remained thus for three minutes. Examination later showed the egg neatly tucked away in the crevice.

PREOVIPOSITION PERIOD

Individuals begin depositing eggs as soon as 5 to 7 days after emergence. Oviposition was repeatedly observed to have occurred this soon after emergence during hot midsummer weather, when large numbers of eggs were being obtained. The length of the preoviposition periods of 10 females are given in Table 1. The length varies greatly with the season of the year, and in these cases in Table 1 it ranged from 8 days as a minimum to 207 days as a maximum. Beetles emerging in the fall at Washington, D. C., rarely oviposit before the following April; females emerging in the spring at Washington normally have a preoviposition period of from 10 to 14 days, depending upon the prevailing temperature.

LENGTH OF OVIPOSITION PERIOD

As indicated by the data of Table 1, the oviposition period is quite long, ranging from about two to five months. The two longest periods were each of 145 days' duration—from March 22 to August 14 in one case, and from April 1 to August 24 in the other.

RATE OF OVIPOSITION AND TOTAL NUMBER OF EGGS DEPOSITED

The number of eggs normally produced on the days when oviposition took place was from 1 to 4, as indicated by the data of Table 2. Occasionally as many as 6 or 8 eggs were laid by a single female in 24 hours. The total number of eggs laid by individuals ranged from 45 to 285 (Tables 1 and 2).

THE EGG

The egg is white, shiny, and elongate-oval in form. In length it measures 0.83 mm. to 0.88 mm. and in width 0.25 mm. (fig. 3, A). The young embryo when fully developed nearly fills the eggshell, which becomes wrinkled and undulates with the movements of the embryo. When ready to break from the shell, the embryo sets in motion a series of undulations from caudad forward, the head and prothorax seeming to swell up at the end of each undulation, stretching the shell of the egg to the bursting point. Finally the shell breaks at the head and the undulating movements carry the young larva gradually clear of it.



FIG. 3.—A, eggs of *Oryzaephilus surinamensis*, $\times 16$ (photographed on No. 10 XX flour bolting cloth); B, white cocoons, greatly enlarged, formed by the larval parasite *Neoscleroderma tarsalis* (Ashm.).

TABLE 2.—Daily oviposition records of 10 female of *Oryzaephilus surinamensis*

Date	Number of eggs laid by individual No.—									
	1	2	3	4	5	6	7	8	9	10
1922										
Mar. 18.....	0	0	0	0	1	0	0	0	0	0
19.....	0	0	0	0	2	0	0	0	0	0
20.....	0	0	0	0	2	0	0	0	0	0
21.....	0	0	0	0	1	0	0	0	0	0
22.....	0	0	0	0	1	0	0	1	0	0
24.....	0	0	0	0	1	1	1	1	0	0
26.....	1	0	0	0	1	2	1	2	0	0
27.....	0	0	0	0	1	1	1	1	0	0
28.....	1	0	0	0	1	2	1	1	0	0
29.....	0	0	0	0	1	2	1	2	0	0
30.....	2	0	0	0	1	0	1	2	0	0
31.....	2	0	0	0	1	0	0	1	0	0
Apr. 1.....	1	0	1	1	2	0	2	2	0	0
2.....	1	1	1	0	2	1	1	2	1	0
3.....	2	0	2	2	1	0	1	0	0	0
4.....	2	1	0	2	2	2	2	2	1	0
5.....	2	2	0	2	1	0	2	0	0	0
6.....	2	2	2	2	2	2	1	1	0	0
7.....	2	2	2	2	2	1	2	1	1	0
8.....	3	1	1	1	1	0	1	0	0	0
9.....	2	0	2	1	2	0	2	0	0	0
10.....	3	1	3	2	3	2	2	2	1	0
11.....	2	2	2	1	2	1	2	0	0	0
12.....	2	2	1	2	2	0	2	0	1	0
13.....	3	2	2	2	2	1	1	0	1	0
14.....	4	2	2	1	2	1	3	1	0	0
15.....	3	0	2	2	0	0	0	0	0	0
16.....	1	0	2	2	0	1	1	0	1	0
17.....	1	0	2	2	0	1	1	2	1	0
18.....	0	1	3	2	0	2	2	1	1	0
19.....	0	1	3	1	0	1	1	0	0	0
20.....	0	0	2	1	0	0	1	0	0	0
21.....	0	0	1	0	0	0	0	2	0	0
22.....	0	0	1	0	0	0	0	0	0	0
23.....	0	0	1	0	0	2	0	0	0	0
24.....	0	0	2	0	0	0	1	0	0	0
25.....	2	0	1	0	0	2	1	2	0	0
26.....	1	0	1	2	0	0	3	0	0	0
27.....	2	0	2	2	0	0	4	0	2	0
28.....	2	0	1	3	0	0	3	2	1	0
29.....	3	0	1	1	0	0	3	0	1	0
30.....	4	0	2	4	0	1	4	2	0	0
May 1.....	5	0	3	3	1	2	4	2	0	0
2.....	4	0	3	2	0	1	3	2	0	0
3.....	4	0	2	3	0	1	3	2	0	0
4.....	2	2	5	2	2	0	4	2	0	0
5.....	3	2	5	1	2	0	5	1	1	0
6.....	0	1	2	0	3	0	5	1	0	0
7.....	1	1	3	1	4	0	6	1	0	0
8.....	1	1	3	1	3	1	6	1	0	0
9.....	0	1	3	2	2	0	4	1	1	0
10.....	1	0	3	1	3	1	4	1	1	0
11.....	2	1	3	3	2	1	6	2	2	0
12.....	3	1	3	3	2	1	6	3	1	0
13.....	2	1	3	3	2	0	6	3	1	0
14.....	2	0	3	3	2	1	6	3	1	0
15.....	2	1	3	3	2	1	6	3	1	0
16.....	3	2	1	3	2	2	8	2	1	0
17.....	3	1	1	4	1	1	8	3	1	0
18.....	3	1	3	3	1	1	4	2	2	0
19.....	3	0	4	3	0	1	4	2	2	0
20.....	2	2	2	3	0	1	5	2	2	0
21.....	3	0	3	3	0	1	3	1	0	0
22.....	3	0	1	2	0	1	4	3	1	0
23.....	3	0	3	2	0	1	2	2	1	0

TABLE 2.—Daily oviposition records of 10 females of *Oryzaephilus surinamensis*—Continued

Date	Number of eggs laid by individual No.—									
	1	2	3	4	5	6	7	8	9	10
May 24.....	3	0	3	1	1	1	4	2	0	0
25.....	3	0	3	3	0	1	2	1	2	0
26.....	3	0	3	2	0	1	3	3	0	0
27.....	2	0	1	2	1	1	3	2	1	0
28.....	3	0	3	1	1	1	2	2	1	0
29.....	2	0	3	3	0	1	3	1	1	0
30.....	2	0	2	1	0	1	1	2	0	0
31.....	2	0	3	1	0	1	4	2	1	0
June 1.....	3	0	3	2	0	1	3	3	0	0
2.....	2	0	3	3	0	1	2	2	1	0
3.....	2	0	3	2	0	1	3	2	0	0
4.....	3	0	3	2	0	1	3	2	1	0
5.....	4	0	2	3	0	1	2	2	0	0
6.....	4	3	4	4	0	0	4	4	0	0
7.....	4	3	4	4	0	0	4	4	0	1
8.....	5	0	2	4	0	1	4	0	0	0
9.....	6	0	2	4	2	1	4	0	1	1
10.....	6	0	1	4	0	2	4	2	0	1
11.....	5	1	1	4	0	2	4	1	0	1
12.....	6	2	1	4	0	2	4	2	0	2
13.....	2	2	1	2	0	2	2	0	0	1
14.....	2	0	1	2	0	2	3	0	0	1
15.....	2	2	0	2	0	0	2	0	0	1
16.....	3	2	1	2	1	1	2	1	0	1
17.....	2	2	1	3	0	0	2	2	0	2
18.....	4	0	0	2	0	0	4	2	1	1
19.....	5	0	0	2	0	0	4	1	0	0
20.....	4	0	0	2	0	0	4	1	0	2
21.....	5	2	0	1	0	0	2	2	1	1
22.....	4	1	0	2	0	0	2	1	1	1
23.....	3	2	0	1	0	0	1	1	0	1
24.....	3	2	0	1	0	0	1	1	0	1
25.....	3	2	0	1	0	2	1	1	0	1
26.....	3	2	0	1	0	1	1	1	0	1
27.....	0	3	0	2	0	2	1	4	0	1
28.....	0	2	0	2	0	1	1	4	0	1
29.....	0	0	0	3	0	2	0	1	0	1
30.....	0	0	0	4	0	2	1	2	0	1
July 1.....	0	0	0	5	0	2	0	1	0	1
2.....	0	0	0	4	0	2	1	5	0	6
3.....	0	0	0	4	0	2	1	6	0	6
4.....	0	0	0	1	0	0	0	4	0	2
5.....	0	0	0	1	0	0	1	3	0	3
6.....	0	0	0	1	0	2	0	1	0	3
7.....	0	0	0	1	0	0	0	1	0	3
8.....	0	0	0	1	0	0	0	2	0	3
9.....	0	3	0	1	2	0	0	1	0	3
10.....	0	2	0	2	2	0	0	2	0	3
11.....	0	4	0	3	2	0	0	1	0	4
12.....	0	3	0	3	1	0	1	3	0	4
13.....	0	2	0	2	0	0	0	2	0	5
14.....	0	1	0	2	0	2	0	2	0	5
15.....	0	2	0	1	0	0	0	1	0	2
16.....	0	2	0	1	1	0	0	1	0	2
17.....	0	2	0	1	0	1	0	1	0	2
18.....	0	2	0	3	0	0	0	3	1	4
19.....	0	2	0	3	0	1	1	1	0	3
20.....	0	0	0	1	0	0	3	4	0	4
21.....	0	0	0	1	0	0	3	2	0	4
22.....	0	1	0	1	2	0	0	1	0	6
23.....	0	1	0	1	2	0	0	1	0	7
24.....	0	1	0	1	2	0	0	2	0	7
25.....	0	0	0	2	0	0	0	1	0	5
26.....	0	0	0	2	0	0	0	1	0	5
27.....	0	0	0	1	1	0	1	1	0	3

TABLE 2.—Daily oviposition records of 10 females of *Oryzaephilus surinamensis*—Continued

Date	Number of eggs laid by individual No.—									
	1	2	3	4	5	6	7	8	9	10
July 28.....	0	0	0	1	1	0	0	2	0	3
29.....	0	0	0	0	0	0	0	0	0	5
30.....	0	0	0	0	1	0	0	1	0	5
31.....	0	0	0	1	0	0	0	0	0	5
Aug. 1.....	0	0	0	0	1	0	1	2	0	4
2.....	0	0	0	0	1	0	0	0	0	4
3.....	0	3	0	0	1	0	0	2	0	4
4.....	0	2	0	0	1	0	0	1	0	5
5.....	0	2	0	0	1	0	0	0	0	3
6.....	0	2	0	0	1	0	1	3	0	4
7.....	0	2	0	0	1	0	1	2	0	3
8.....	0	3	0	0	0	0	0	1	0	4
9.....	0	3	0	0	0	0	1	0	0	4
10.....	0	1	0	0	0	0	2	0	0	2
11.....	0	1	0	0	0	0	1	1	0	3
12.....	0	0	0	0	0	0	0	4	0	1
13.....	0	0	0	0	0	0	1	0	0	1
14.....	0	0	0	0	0	0	1	1	0	1
15.....	0	0	0	0	0	0	0	0	0	3
16.....	0	0	0	0	0	0	0	1	0	2
17.....	0	0	0	0	0	0	0	0	0	3
✓ 18.....	0	0	0	0	0	0	0	0	0	4
19.....	0	0	0	0	0	0	0	0	0	3
20.....	0	0	0	0	0	0	0	0	0	3
21.....	0	0	0	0	0	0	0	0	0	2
22.....	0	0	0	0	0	0	0	0	0	3
23.....	0	0	0	0	0	0	0	0	0	3
24.....	0	0	0	1	0	0	0	0	0	3
25.....	0	0	0	0	0	0	0	0	0	3
26.....	0	0	0	0	0	0	0	0	0	3
27.....	0	0	0	0	0	0	0	0	0	3
28.....	0	0	0	0	0	0	0	0	0	4
Total.....	226	117	167	239	103	96	285	216	45	235

LENGTH OF EGG STAGE

The length of the egg stage varies with the temperature, as is indicated by the data of Table 3. In midsummer, with the mean average temperatures ranging from about 80° to 85° F., the eggs hatch in from 3 to 5 days after deposition. In cool spring and fall weather, with the mean average temperatures ranging from 73° to 68°, the eggs hatch in from 8 to 17 days.

TABLE 3.—Incubation period of egg of *Oryzaephilus surinamensis*

No.	Date egg was laid	Date egg was hatched	Incubation period	Mean average temperature for period	No.	Date egg was laid	Date egg was hatched	Incubation period	Mean average temperature for period
	1921	1921	Days	° F.		1921	1921	Days	° F.
1.....	July 20	July 24	4	82	16.....	Oct. 20	Nov. 3	13	70
2.....	July 21	July 25	4	82	17.....	Oct. 24	Nov. 4	11	70
3.....	July 22	do.	3	82	18.....	Nov. 10	Nov. 22	12	68
4.....	July 23	July 26	3	83	19.....	Nov. 13	Nov. 26	13	68
5.....	July 24	July 27	3	85	20.....	Nov. 18	Nov. 30	12	69
6.....	Aug. 1	Aug. 5	4	80	21.....	Nov. 23	Dec. 6	13	68
7.....	Aug. 2	Aug. 7	5	80					
8.....	Aug. 3	Aug. 8	5	80					
9.....	Sept. 12	Sept. 17	5	79	22.....	1923	1923		
10.....	Sept. 25	Oct. 3	8	73	23.....	Mar. 1	Mar. 14	13	71
					24.....	do.	Mar. 16	15	71
11.....	do.	Oct. 5	10	72	25.....	Mar. 5	Mar. 22	17	70
12.....	Sept. 29	Oct. 9	10	70	26.....	do.	Mar. 21	16	70
13.....	do.	Oct. 11	12	70		Mar. 7	Mar. 23	16	70
14.....	Oct. 2	Oct. 14	12	69					
15.....	Oct. 15	Oct. 27	12	70					

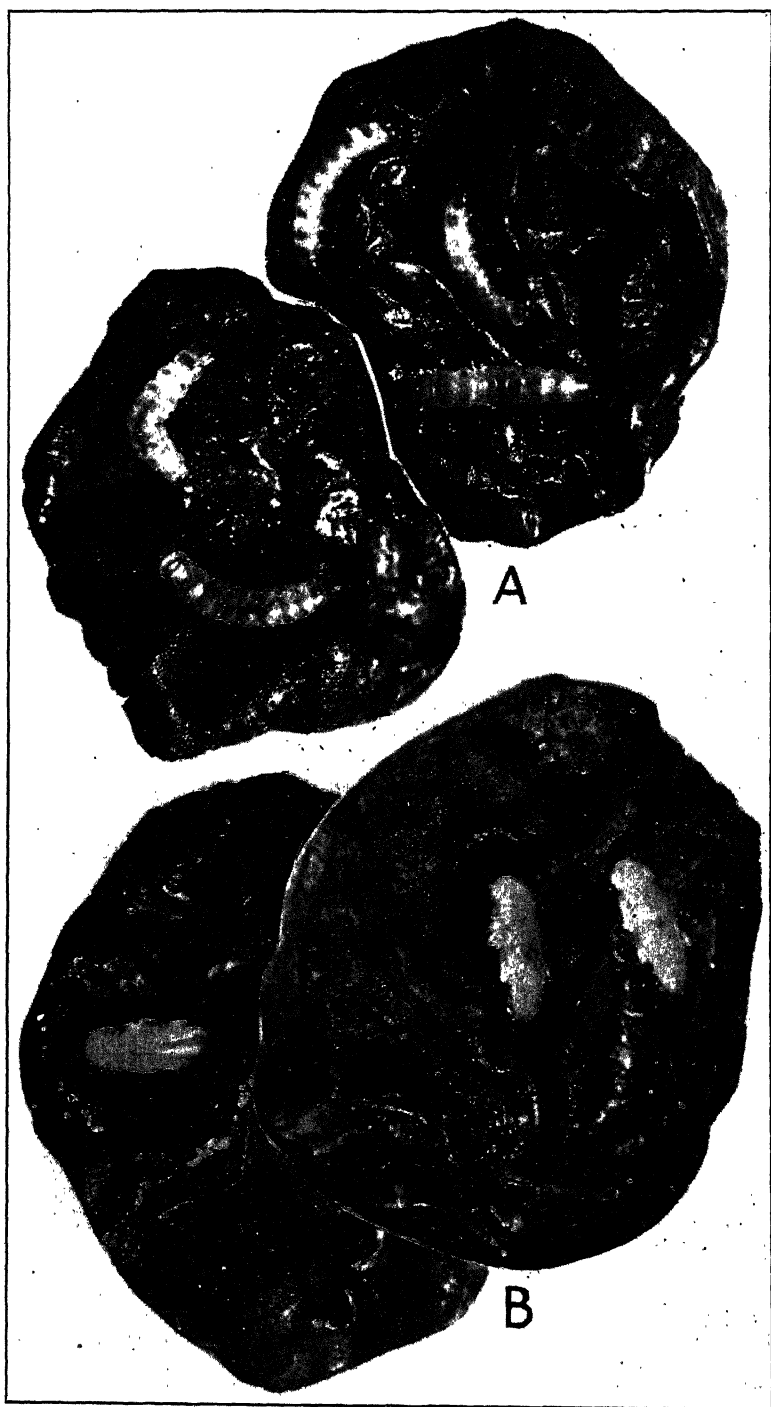


FIG. 4.—*Oryzaephilus surinamensis*: A, Five well-grown larvae on small seedless raisins; B, three pupae on seedless raisins. All greatly enlarged

THE LARVA

The larva is white when first hatched, and its movements are slow and uncertain. It soon gains strength and becomes active, however, and begins to attack the available food. Its body acquires a pale yellowish color, with darker bands on the dorsal surface of the thoracic and abdominal segments. Numerous long hairs adorn these segments. The head capsule is a pale yellowish brown. When first hatched the larva is about 0.80 to 0.90 mm. in length, and the width of the head capsule is 0.24 mm. When fully grown the larva attains a length of from 2.5 to 2.8 mm., and the width of the head capsule is from 0.46 mm. to 0.54 mm.³ (Fig. 4.)

The larva thrives on practically all foodstuffs of vegetable origin, such as corn, wheat, barley, and rice, both ground and unground, and in all their varied forms, such as flour, meal, and breakfast foods; dried foods, nut meats, copra, etc. It is free-living and active, and does not always confine its feeding to one spot, but nibbles here and there as fancy dictates. It is apparently unable to feed on whole grain unless the grain is quite soft. It is therefore frequently associated with other insects, such as the rice weevil, which bore into whole grain and thus afford entry to the weaker species.

LENGTH OF LARVAL STAGE

The length of the larval stage as determined at Washington, D. C., varied considerably, and was affected chiefly by the temperature. Data bearing on larval life will be found in Table 4. During the spring the period from hatching to pupation ranged from about 4 to 7 weeks, whereas in midsummer this period was about 2 weeks, 12 days being the shortest larval period recorded. With the approach of cooler weather in the fall, the period again lengthened until during the winter months, when the laboratory was heated only during the day, the period was about 8 to 10 weeks.

The number of molts varied (Table 4.) A majority of the larvae observed molted three times, a few molted four times, and quite a number molted but twice. Those individuals that molted four times were all reared during the fall and winter, when development was slow and the larval period quite long.

³ A technical description of the typical silvanid larva has been given by Böving (6, pp. 201-203).

TABLE 4.—Life-history data on 30 individuals of *Oryzaephilus surinamensis*

No.	Date egg was laid	Date hatched	Length of egg period	Date of first molt	Length of first larval stage	Date of second molt	Length of second and larval stage	Date of third molt	Length of third larval stage	Date of fourth molt	Length of fourth larval stage	Date of beginning of prepupal stage	Length of last pupal stage	Date of pupation	Length of prepupal stage	Date adult emerged	Length of pupal period	Days	° F.	Mean average temperature for 1 period
1	1921 July 19	1921 July 23	4	July 30	7	1921 Aug. 3	4	Aug. 7	4	1921 Aug. 9	9	Aug. 6	5	Aug. 10	1	Aug. 20	10	18	32	80
2	1921 July 20	1921 July 24	4	do.	6	Aug. 1	2	Aug. 4	2	Aug. 6	8	Aug. 6	5	Aug. 10	2	Aug. 16	10	18	32	81
3	1921 July 21	1921 July 25	4	July 30	4	do.	3	Aug. 3	3	Aug. 5	4	Aug. 5	4	Aug. 9	2	Aug. 13	6	13	27	80
4	1921 July 22	1921 July 26	4	July 30	4	do.	2	Aug. 5	4	Aug. 8	8	Aug. 8	3	Aug. 9	1	Aug. 17	8	13	25	80
5	do.	1921 July 27	4	do.	3	Aug. 3	4	Aug. 7	4	Aug. 9	10	Aug. 9	2	Aug. 10	1	Aug. 16	6	14	24	81
6	1921 July 25	1921 July 28	3	July 31	3	Aug. 4	4	Aug. 8	4	Aug. 10	10	Aug. 10	2	Aug. 11	1	Aug. 19	8	14	25	80
7	1921 July 26	1921 July 30	4	Aug. 3	4	Aug. 7	4	Aug. 11	4	Aug. 12	12	Aug. 12	3	Aug. 13	2	Aug. 20	8	13	25	80
8	1921 July 27	do.	5	do.	4	do.	4	Aug. 11	4	Aug. 12	12	Aug. 12	2	Aug. 13	2	Aug. 19	8	12	23	81
9	1921 July 27	do.	3	Aug. 5	4	Aug. 10	5	Aug. 12	5	Aug. 13	13	Aug. 13	2	Aug. 14	4	Aug. 22	6	15	24	79
10	1921 July 31	1921 Aug. 3	3	Aug. 7	4	Aug. 12	5	Aug. 15	5	Aug. 17	17	Aug. 17	5	Aug. 19	2	Aug. 28	10	16	29	78
11	Aug. 1	Aug. 5	4	Aug. 9	4	Aug. 13	4	Aug. 17	4	Aug. 19	19	Aug. 19	2	Aug. 20	1	Aug. 31	11	15	30	78
12	Sept. 12	Sept. 17	5	Sept. 25	8	Sept. 30	5	Oct. 4	5	Oct. 9	9	Oct. 9	2	Oct. 10	3	Oct. 25	13	25	43	73
13	Sept. 27	Oct. 6	9	Oct. 12	6	Oct. 28	16	Nov. 9	12	Nov. 25	16	Dec. 9	14	Dec. 14	5	Jan. 2	19	69	97	69
14	Sept. 28	Oct. 9	11	Oct. 19	10	Oct. 29	10	Nov. 5	7	Nov. 20	15	Nov. 28	8	Dec. 1	3	Dec. 20	19	53	83	69
15	Sept. 29	Oct. 11	12	Oct. 22	11	Oct. 30	8	Nov. 10	11	Nov. 25	15	Nov. 29	19	Dec. 3	4	Dec. 21	18	53	83	69
16	Sept. 30	Oct. 12	12	do.	10	Nov. 2	11	Nov. 14	12	Dec. 1	17	Dec. 22	21	Dec. 26	4	Jan. 16	21	75	108	69
17	Oct. 15	Oct. 27	12	Nov. 4	8	Nov. 18	14	Nov. 28	10	Dec. 8	10	do.	14	Dec. 29	7	Jan. 17	19	63	94	69
18	1923 Mar. 1	1923 Mar. 16	15	Mar. 29	13	Apr. 8	10	Apr. 17	9	Apr. 26	10	Apr. 26	6	Apr. 25	5	May 9	14	40	69	70
19	Mar. 19	Mar. 19	16	Apr. 2	14	Apr. 10	8	Apr. 19	9	Apr. 26	10	Apr. 26	6	May 1	5	May 16	15	43	74	70
20	Mar. 5	Mar. 21	16	Apr. 5	15	Apr. 12	7	Apr. 22	10	May 1	10	May 4	12	May 8	4	May 20	12	43	76	70
21	do.	do.	16	Apr. 3	13	Apr. 10	7	do.	12	May 1	10	May 4	9	May 5	4	May 17	12	45	73	70
22	Mar. 7	Mar. 23	16	Apr. 5	13	Apr. 14	8	May 11	8	May 20	10	May 24	10	Apr. 28	4	May 14	16	36	68	73
23	Apr. 3	Apr. 17	13	Apr. 25	13	May 7	6	May 11	8	May 20	10	May 24	10	Apr. 28	2	May 14	16	36	68	73
24	do.	do.	14	Apr. 22	7	May 1	7	May 9	8	May 20	10	May 24	10	Apr. 28	3	May 30	12	36	68	73
25	Apr. 6	Apr. 19	13	Apr. 27	8	May 5	8	May 13	8	May 20	10	May 24	7	May 23	3	June 2	10	34	57	73
26	Apr. 5	Apr. 18	13	Apr. 26	8	May 8	12	May 16	11	May 19	19	May 19	11	May 21	2	June 3	10	33	56	73
27	Apr. 6	Apr. 19	13	Apr. 27	8	May 9	8	do.	7	May 24	24	May 24	6	do.	2	June 3	8	37	58	73
28	Apr. 7	Apr. 20	13	May 3	13	May 9	6	do.	11	May 22	22	May 22	8	do.	4	June 3	8	36	57	73
29	do.	do.	13	Apr. 28	8	May 6	8	May 12	6	May 24	24	May 24	10	May 18	2	May 31	10	34	57	73
30	do.	do.	13	do.	8	May 5	7	May 12	6	May 15	15	May 15	10	May 15	3	May 28	10	28	51	73

THE PUPA

When the larva completes its growth it usually constructs a rude pupal cell or cocoon with particles of seeds or other foodstuffs, fastening them together with an oral secretion. At other times no pupal cell is made. In both cases the larva attaches itself by the anal end to some solid object. After a short prepupal period, during which time it is more or less quiescent, it transforms to the pupal form. (Fig. 3, B.) The pupa remains attached by its caudal end to the cast larval skin. Blissom (5) described this transformation in 1849.

For the individuals in Table 4 the prepupal period ranged from 1 to 7 days, and the pupal period from 6 to 21 days. (Table 5.)

TABLE 5.—Effect of temperature on the length of the pupal stage of *Oryzaephilus surinamensis*, as indicated by 30 individuals

No.	Date pupated	Date adult emerged	Length of pupal stage	Mean average temperature for period	No.	Date pupated	Date adult emerged	Length of pupal stage	Mean average temperature for period
			Days	° F.				Days	° F.
1.....	1921 July 31	1921 Aug. 7	7	81	16.....	1921 Dec. 26	1922 Jan. 16	21	68
2.....	Aug. 7	Aug. 13	6	77	17.....	Dec. 29	Jan. 17	19	68
3.....	Aug. 8	Aug. 16	8	76					
4.....	Aug. 10	Aug. 19	9	76.5	18.....	Apr. 23	May 8	15	72
5.....	Aug. 12	...do....	7	77	19.....	Apr. 25	May 9	14	72
					20.....	Apr. 27	May 13	16	72
6.....	Aug. 13	Aug. 20	7	78					
7.....	...do....	Aug. 19	6	78	21.....	Apr. 28	May 14	16	72
8.....	Aug. 16	Aug. 22	6	78	22.....	May 1	May 16	15	73
9.....	Aug. 19	Aug. 27	8	76	23.....	May 5	May 17	12	73
10.....	Oct. 12	Oct. 25	13	71	24.....	May 8	May 20	12	73
					25.....	May 18	May 28	10	75
11.....	Oct. 20	Nov. 2	13	70					
12.....	Nov. 30	Dec. 17	17	66	26.....	May 22	May 31	9	77
13.....	Dec. 1	Dec. 20	19	69	27.....	...do....	June 2	11	77
14.....	Dec. 3	Dec. 21	18	69	28.....	May 23	...do....	10	77
		1922			29.....	May 26	June 3	8	80
15.....	Dec. 14	Jan. 2	19	69	30.....	...do....	...do....	8	80

LENGTH OF LIFE CYCLE

The effect of temperature on the length of time required for the individual to pass through the egg, larval, and pupal stages is indicated by the following data derived from Table 4. In warm summer weather, when the mean average temperature for the period of development was 81° F., three individuals required 22, 24, and 27 days to pass through the egg, larval, and pupal stages. When the mean average temperature was 80° five other individuals required 23, 25, 25, and 32 days. In the spring, when the mean average temperature was 70°, five other individuals required 68, 73, 76, 74, and 69 days. The longest period noted was 108 days (No. 16, Table 4), the mean average temperature for the period (September 30 to January 16) being 69°. Four other specimens required 83, 83, 94, and 97 days, at the same temperature. With the life of the adult ranging from a few days to 3 years and 3 months, the length of one generation varies greatly. Inasmuch as the preoviposition period has been found to range from 5 to 207 days and the period from egg to adult from 22 to 108 days, it seems safe to state that the cycle from egg to egg may range from 27 to 315 days. In the vicinity of Washington, D. C.,

there are normally from four to five generations a year, when one considers a generation as covering the period from the time of hatching to the laying of the first eggs by the emerging adult. In subtropical and tropical climates the breeding is undoubtedly continuous.

PARASITES

As far as the writers are aware, the only parasite of the saw-toothed grain beetle is a hymenopteran described by Ashmead (1, p. 45) as *Ateleopterus tarsalis* from specimens reared from larvae of *Oryzaephilus surinamensis* infesting raisins. He stated that adults had been reared in Indiana from *O. surinamensis* infesting grain. Washburn (34, p. 205) and Brues (7, p. 610) refer to this parasite as *Neoscleroderma tarsalis* (Ashm.) in their accounts of the Hymenoptera of Minnesota and Connecticut, respectively, but neither gives further information. The writers have reared this parasite from larvae of *O. surinamensis* infesting raisins at Washington, D. C., and in 1922 they received specimens reared in Texas by T. Remy. The cocoons spun by the larvae of the parasite are illustrated in Figure 2, B. It is doubtful whether this parasite is of practical value, except, perhaps, in the case of natural control, when its host is infesting grain and stock feeds held in storage for unusually long periods.

CONTROL MEASURES

A study with the object of devising control measures for the saw-toothed grain beetle is in progress, and it may be well to report some of the findings here.

STARVATION.—The beetles are not very resistant to starvation; adults kept without food in midsummer were all dead within five days.

HIGH AND LOW TEMPERATURES.—The insect is fairly resistant to low temperatures in all stages except the egg stage. De Ong (12, p. 446; 13, p. 74) stated that a three months' exposure at 10° to 36° F. would kill the larvae, pupae, and adults. The writers found that a small percentage of adults and larvae survived an exposure for a period of three weeks within a temperature range of 30° to 35° F. Exposure within a temperature range of 20° to 25° killed all stages in one week, and exposure to 0° to 5° killed all stages in one day.

All stages succumb readily to heat. Goodwin (19) found that a temperature of 111° to 113° F. was fatal to larvae, pupae, and adults, although he did not state what length of exposure was necessary. Dean (10, p. 163) has reported that no grain pest (including the saw-toothed grain beetle) can withstand a temperature of from 118° to 122° for any considerable length of time. The writers found that exposure for one hour to 125° was fatal to all stages.

FUMIGANTS.—Hinds (23, pp. 20-21) found that the saw-toothed grain beetle lived for about two hours in an atmosphere saturated with carbon disulphide at a temperature of 90° F. The writers (2) have indicated that the ethyl acetate-carbon tetrachloride mixture can be used successfully in killing this pest. As a rule, a heavier-than-air gas is more satisfactory in fumigating infested grain, grain products, and nut meats. Hydrocyanic-acid gas is used frequently and with success under certain conditions calling for no great penetration of infested bulks. Hamlin and Benton (22) have recently established the

practicability of fumigating stemmer trash at raisin-packing plants in California with hydrocyanic-acid gas generated from calcium cyanide flakes.

Bioletti (4, p. 189) recommends the following method for the disposal of this infested trash:

By modifying the shape of the receptacle which receives the screenings, including insects, all the beetles could be caught and destroyed. The modification consists of an over-lapping removable border furnished with a pad saturated with coal-oil or phenol to prevent the escape of the beetles. At intervals this receptacle is emptied and the insects destroyed.

HERMETICALLY SEALING AND VACUUM.—Dendy (11, p. 11) has found that hermetically sealing 100 adult beetles in a 15 c. c. capacity test tube three-fourths filled with crushed wheat for two days at 31° C. (87.8° F.) resulted in their death. The writers (3) found that adults kept in a vacuum of 29 inches for seven hours were killed.

SUMMARY

The saw-toothed grain beetle, *Oryzaephilus surinamensis* Linné, is one of the best known of the cosmopolitan grain pests. It attacks in both its larval and adult stages all food of vegetable origin, especially grain and such grain products as flours, meals, breakfast foods, stock and poultry feeds, and copra, nut meats, candies, and dried fruits.

No detailed study of this pest seems to have previously been made. Adult beetles have been found to live for more than 3 years. The majority of ovipositing females live from 6 to 10 months. The length of the preoviposition period ranges from 5 to 8 days during the season most favorable for breeding, to 207 days when climatic conditions are less favorable. The total number of eggs laid by an individual has been found to range from 45 to 285.

Eggs hatched in from 3 to 5 days in midsummer. In cooler weather hatching may not have taken place until 8 to 17 days after deposition. Larvae required about 12 days for development, under favorable midsummer conditions. In the spring from about 4 to 7 weeks were required to pass the period from hatching to pupation. Under less favorable conditions larvae have required 10 weeks for development. Larvae molt from two to four times, the majority molting three times. Data presented indicate a prepupal period of 1 to 7 days, and a pupal period of 6 to 21 days.

Under the most favorable weather conditions, the egg, larval, and pupal stages may be passed in as few as 22 days. The longest period required for the same development was 108 days, when the mean average temperature for the period was 69° F. The life cycle from egg to egg may range from 27 to 315 days. At Washington, D. C., there are four or five generations annually; in subtropical and tropical climates breeding is undoubtedly continuous.

As far as the writer is aware, the only known parasite of the saw-toothed grain beetle is a small cocoon-making hymenopteron, *Neoscleroderma tarsalis* (Ashm.).

In the adult stage the saw-toothed grain beetle is quite resistant to fumigants, but it can be killed by fumigation with hydrocyanic-acid gas, carbon disulphide, ethyl acetate-carbon tetrachloride mixture, or any other effective fumigant. Although an exposure of from 0° to 5°

F. for one day will kill all stages, adults and 'arvae withstood for three weeks a temperature ranging from 30° to 35°. An exposure for one hour at 125° has killed all stages. A vacuum of 29 inches continued for seven hours has killed adults.

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CORN ROOT ROT—A SOIL-BORNE DISEASE¹

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INTRODUCTION

The present situation in regard to the corn root-rot problem is well summarized by Holbert and others in the following statements by them in a recent publication (4, p. 243):² "The corn root, stalk, and ear rot diseases are a group of diseases seriously affecting the corn crop. For brevity, these diseases are sometimes called 'corn-rot diseases,' and frequently simply 'corn root rot.' However, at the outset it must be realized that 'corn root rot' is not one disease, but several diseases, some of which do not result in any rotting of either roots or stalks." Although it is realized that the problem is complicated by the fact that there are several diseases, sufficient attention has not been given by plant pathologists to an attempt to separate the various diseases concerned and to properly evaluate them.

Confusion has arisen mainly from the fact that in the earlier studies of the problem the relation between poor seed and seedling blights was emphasized. These seedling blights, if they did not kill the plants, stunted them so seriously that they were at a disadvantage throughout life, although they may have largely recovered from the initial injury. These earlier studies centered attention on seed-borne pathogens, nearly to the exclusion of a study of the organisms actually associated with the diseased corn roots. In much of the literature on corn root rot it appears that the delayed development following seedling injury has been considered as typical corn root rot. If these effects of seedling blight are to be considered corn root rot, the fact should be recognized. If, however, there is a true corn root rot other than seedling blight, the two diseases should be recognized clearly and a distinction should be made between them in future literature.

That there is a disease (or group of diseases) which should be known as corn seedling blight is evident from the abundant literature on seed selection and the corn root rot problem. The work of Hoffer, Holbert, and others shows clearly that seed known to be infected with *Gibberella saubinetii*, *Diplodia zeae*, or possibly others of the common corn ear pathogens, gives a crop inferior to one produced from apparently healthy seed. It has been shown that the diseased seeds often produce weak plants which may die prematurely or may be weak throughout their lives. This fact has been recognized for a long time by many careful farmers who have made it a practice to discard all ears which are likely to be affected with any of the true ear-rot organisms (*Diplodia zeae*, *Gibberella saubinetii*). By ear selection at harvest time and by curing it is possible to obtain ears for seed in which the factor of seedling blight is almost negligible.

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² Reference is made by number (italic) to "Literature cited," p. 475.

This does not mean that ears free from seed-borne organisms may be obtained by such means, but that seedlings are produced sufficiently vigorous to develop in spite of the presence of such common organisms as *Fusarium moniliforme* and *Rhizopus* sp., which in weak corn appear to be able to cause seedling blight by attacking the surface of the scutellum and thus cutting off the food supply of the young plant.

The elimination of the factor of seedling blight does not, however, in the writers' observation, in any way control root rot which appears later in the season. About the time of ear formation in Kentucky it is difficult, on corn grown in some long-cultivated fields, to find more than a few live roots, the plants appearing to subsist on moisture and plant food drawn from the soil largely through dead roots and new roots thrown out after rainy periods. On continuous-corn plots on the Kentucky Agricultural Experiment Station farm, on land kept in a high state of fertility, this condition is much more prominent than in plots in the longer rotations; the plants may make a vigorous early growth, but when nearing maximum growth they may show marked symptoms of root injury, as evidenced by wilting, curling, and premature death of the leaves, while the corn in near-by rotation plots with actually lower soil moisture may not be wilting. This condition appears to the writers to be that which should be recognized as corn root rot in contrast to seedling blights and poor development due to adverse soil conditions.

With regard to the identity of the supposed causes of corn root rot as it is generally understood, there seems to be some unanimity of opinion in the recent literature. Manns and Phillips (6), following a review of recent literature, state:

The literature as here reviewed shows that investigators have determined upon four different organisms as the principal ones associated with corn-rot diseases. These organisms are *Fusarium moniliforme* Sheldon; *Gibberella saubinetii* (Mont.) Sacc.; *Diplodia zeae* (Schw.) Lév., and *Cephalosporium sacchari* Butler. It is known that several of these organisms inhibit germination and appear to be active factors in the production of stalk and ear rots. Their importance as factors in causing root rot, however, is still in dispute. It is even questioned whether soil reaction is not the real factor predisposing corn to attacks of these so-called seedling and root-rot diseases.

Tehon (8) states that "Root and stalk rots of corn are chiefly caused by the fungi *Fusarium moniliforme*, *Diplodia zeae*, and *Gibberella saubinetii*. The degree of injury done by these organisms is dependent upon the relative susceptibility of the corn plant in the various stages of its development, and the effects of the attack appear as rotted roots, barren stalks, or poorly filled ears."

Holbert (4) and his coworkers apparently are in accord with these statements in regard to the causes of corn root rot, as their principal line of attack has been a study of the seed-borne organisms and their effect on the seedling plant and its subsequent development. Their work deals primarily with *Gibberella saubinetii* and *Diplodia zeae* and the effect of the various environmental factors on development of plants from seed infected and not infected by these organisms. They recognize the importance of soil infection in the problem, for they say: "Of the environing factors affecting the development of the different corn-rot diseases under discussion in this bulletin, the problem of *crop rotations* and *crop sequence* takes a place second to no other under conditions existing at the present time in central

Illinois." That they consider that *G. saubinetii* plays an important part in soil contamination is suggested by the statement (4, p. 323) that "In the experiment at Bloomington in 1920 there was a difference of 14.1 bushels in acre yield between corn from good seed and from scutellum-rotted seed where corn followed clover, but in the same field where corn followed badly scabbed spring wheat the difference was 33.1 bushels."

In the Plant Disease Reporter (9) the cause of corn root rot is given as *Gibberella saubinetii*.

It is the object of this paper to point out the fact that there is a root disease of corn distinct from seedling blight and its after effects, and that the organisms commonly considered as etiologic factors in corn root rot probably are not concerned in the problem.

SOIL-STERILIZATION EXPERIMENTS

EXPERIMENT 1

For this experiment two lots of soil were collected April 7, 1924, one from a field in corn continuously from 1911 to 1923, and the other from a hardwood forest near by on the same type of soil, which may be considered virgin. The samples were collected and the work done in the spring of 1924. The soils were treated, or not, as follows: (1) Virgin soil untreated; (2) virgin soil steamed at 20 pounds pressure for one hour; (3) corn soil untreated; (4) corn soil to which a mineral fertilizer was added;² (5) corn soil steamed at 20 pounds pressure for one hour; (6) corn soil treated with formaldehyde;³ and (7) corn soil steamed and fertilized.

Uniform plants were not used throughout, seed-borne organisms being eliminated⁴ from the plants used in the virgin soil and the soils which were partially sterilized, but they were not eliminated from the plants in the natural corn soil. All series were run in duplicate.

The average dry weights of the roots and tops of the two plants per pot in the two series, one of which was removed on the eightieth day and the other on the ninety-third day, are given in the following tabulation:

	Tops	Roots
	Grams	Grams
Virgin soil, untreated.....	190.5	27.7
Virgin soil, steamed.....	214.9	13.5
Corn soil, untreated.....	52	14.1
Corn soil, fertilized.....	125.7	28.3
Corn soil, steamed.....	214.2	29.9
Corn soil, formaldehyde.....	145.3	18.5
Corn soil, steamed and fertilized.....	203.7	18.1

² The chemicals used and the rate per acre were as follows: Acid phosphate, 500 pounds; lime, 200 pounds; KCl, 200 pounds; and nitrate of soda, 200 pounds.

³ The soil was saturated with a solution of 1 c. c. of 40 per cent formaldehyde to 350 c. c. of water heated to 90° C. The pots were allowed to stand three days and were then emptied and the soil aerated, dried until it worked well, and then sieved and replaced in the pot.

⁴ The seeds were treated in water 24 hours, followed by 11 minutes soaking in hot water at 55° C. and then 15 minutes in 1:1000 mercuric chloride solution, and washed three times in sterile water (Sherbakoff's method (7)). They were then planted in sterile sand. When rooted, the plants were removed from the sand, the endosperm and seed coats were removed, and the roots were washed in a weak calcium hypochlorite solution. The endosperm and seed coats were removed from the untreated seeds used in the natural corn soil in the same way, but were replaced with the seedling. The plants were grown in 4-gallon pots; in the bottom of which a small flowerpot was inverted. A glass tube which extended above the surface of the soil was inserted in the hole in the pot and water was introduced through this tube.

The results of this experiment may be summarized as follows:

The roots of 80-day-old plants grown in virgin soil steamed and unsteamed were clean when washed out, showing no lesions; the 93-day-old roots in unsteamed virgin soil showed an occasional small lesion when washed out.

The roots grown in unsteamed, unfertilized, and fertilized corn soil were brown throughout and were very much decayed, while in the same soil steamed they were white and healthy throughout (fig. 1).

The roots from corn soil treated with formaldehyde were clean and white throughout, showing no signs of injury, the temporary root system being still alive and healthy.

The application of fertilizers to the unsteamed corn soil did not decrease the amount of injury to the roots although the root systems in this soil were practically twice as large as those grown in unsteamed unfertilized soil, on the basis of dry weights.

Soil sterilization in the presence of abundant fertility seems to result in the development of a smaller root system (but larger tops) than in unsteamed soil, whether the unsteamed soil is infected with root rot or not.

As the results obtained can not with certainty be attributed to soil infection, but might be explained on the basis of seed infection, these tests will be used only to indicate the importance of corn root rot in the economy of the corn plant. However, the writers believe the results were not due to seed infection, although *Fusarium moniliforme* and possibly other common seed organisms were present in the untreated seed, yet the growth of the seedlings indicated that they were not affected appreciably by these organisms; and the type of injury obtained in the old corn soil has never been observed by the writers to occur in pot work as a result of planting infected seeds, although many root systems have been examined. Subsequent tests reported in this paper show that the typical corn root rot may be produced when fungus-free seedlings are used.

EXPERIMENT 2

A second experiment was planned on much the same basis as experiment 1, using three soils as follows: (1) Virgin soil as in experiment 1—untreated, steamed, and steamed and fertilized; (2) soil from a continuous-corn plot not manured or fertilized; this was used untreated, steamed, with fertilizers, and with fertilizers and steamed; and (3) continuous-corn soil heavily manured each year; this soil was used steamed and untreated.

In this test, treated seed (as in the previous experiment except that in this case heated to 50° instead of 55° C.) only were used in the virgin soil; in the continuous-corn soil, treated and untreated seed were used, respectively, in both steamed and unsteamed soil, in order to determine the relative importance of seed infection and soil infection; while in the manured corn soil treated seed were used in the steamed soils and untreated in the unsteamed soils.⁶ All tests were run in duplicate in 4-gallon pots equipped as in experiment 1. Thirty-eight pots were included in the original plan of this experiment, but owing to accidents five of them were eliminated.

⁶ Steamed pots were heated in an autoclave for 3¼ hours at 18 to 20 pounds pressure.

Examination of the root systems was made after 104 days (Jan. 9, 1925) by removing the ball of dirt as carefully as possible from the jar and working the dirt away from the roots with the fingers and



FIG. 1.—Roots of corn plants grown in soil from a field continuously in corn from 1911 to 1923. A, roots from untreated soil; B, roots from soil to which fertilizer was added; and C, roots from the same soil steam-sterilized. The roots in C are clean and white in contrast with the severely injured roots grown in the natural soils. As indicated by the relative size of the root systems, the disease in pots A and B was rather slow in developing

then with water. Without going into all details, the following results were obtained:

Steaming and fertilizing had no appreciable effect, as compared with untreated soil, on the development of plants grown in virgin soil. The root systems in all cases were healthy throughout, and the plants made about equal growth.

No differences could be noted in the development of plants in untreated continuous-corn soil when grown from treated and from untreated seeds. In both cases the root systems were nearly completely decayed when examined.

There was evidence in some of the steamed pots that seed treatment and selection of seedlings had not been entirely effective in eliminating seed-borne organisms, as evidenced by the fact that sometimes the cotyledonary internode and the roots coming from it were found to be rotted. The injury, however, was limited to rootlets in an area not more than 2 inches in extent and was very slight. This is the usual effect of the seed-borne organisms most commonly found in well-cured, good seed corn.

The addition of mineral fertilizers to the unmanured continuous-corn soil increased the size of the plants and the extent of the root systems, in both the steamed and the unsteamed pots. It apparently had no effect whatever on the health of the roots in the unsteamed soil, however, as they were nearly completely destroyed when examined (fig. 2).

High fertility maintained by manuring in the manured continuous-corn soil had a marked effect on increasing the number of healthy roots present on these plants, and also on the size of plants. The plants grown in this soil not steamed were as large as those grown in the same soil steamed and in virgin soil, in spite of the fact that the roots were severely rotted (fig. 2). This was evidently due to the fact that the plant made a vigorous start because of available fertility, so that when the roots began to decay the plant was sufficiently vigorous to replace them as they rotted and so maintain its growth. Numerous healthy roots and rootlets were found in the upper 4 inches of soil, while below that point nearly all the roots were dead. In Figure 2 it will be seen that the plant raised in the steamed soil developed 8 permanent roots, whereas that in the diseased soil developed 17. Evidently the difference is not genetic, as the corn used was a three-times selfed strain of Boone Country White which showed a high degree of uniformity in the field and in the pots. The condition of this plant in diseased soil is typical of plants growing in diseased soil in the field, which, when they have attained nearly maximum growth, have lost nearly all of their root systems by decay but have a very marked ability to partially replace the system after rains. This is undoubtedly the reason why the results of corn root rot other than seedling injury due to infected seeds are often so obscure, even though it may readily be seen by pulling a plant that most of the roots are decayed.

From these experiments and from other tests conducted by the writers it may be definitely stated that death of the temporary root system and of the small laterals and larger roots during the growth of a corn plant is not a normal process, as the individual roots, in the absence of injurious organisms, appear to live at least beyond the tasseling stage. The question whether death of the small roots was due to disease or to a natural process within the plant had been raised on several occasions by colleagues during the progress of this and other work on the roots of crop plants.

EXPERIMENT 3

As some difficulty is at times experienced in growing plants in soil which has been steamed at high pressure for extended periods, it was desired to find the lowest temperature and the shortest period of heating which would eliminate the cause of injury to corn roots. An experiment was therefore planned to determine this point. Soil was obtained April 3, 1925, from a field on the experiment station farm which had grown several successive crops of silage corn. After sieving and thoroughly mixing, the soil was placed in 26 one-gallon glazed jars. Pairs of pots were then treated respectively as follows: Steamed at 14 to 16 pounds pressure for 2 hours and the soil immediately spread in a thin layer to cool and then replaced; treated in

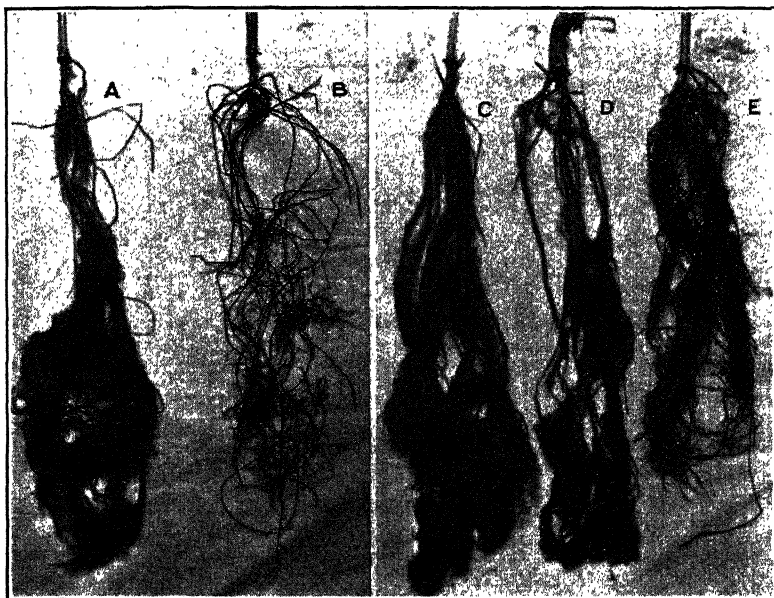


FIG. 2.—A, B, roots of corn plants grown in unfertilized continuous-corn soil to which mineral fertilizers were added for this experiment. The soil in which the plant A grew was steamed, while that in the case of B was not. Fertilization of this soil increased the size of the root systems over those in unfertilized soil, but it did not reduce root-rot injury in the unsteamed fertilized soil. C, D, E, root system of corn plant grown in untreated virgin soil (C), in manured continuous-corn soil steamed (D), and in the same soil unsteamed (E). The roots of C and D are uninjured, but those of E are severely injured, most of the fine laterals being destroyed and the larger roots discolored. More permanent roots developed from the crown of this plant (E) than from the crown of the plant in the steamed soil (D).

the same way as the previous lot but left in the jar after heating; 100° C. for 1 hour; 80° to 85° for 1 hour; 65° to 70° for 1 hour; 65° for 15 minutes after the center of the soil mass had attained this temperature; 40° to 50° for 1 hour; soil air-dried 1 week away from direct sunlight; air-dried for 2 weeks; air-dried for 4 weeks; soil treated with formaldehyde vapor by placing soil on a wire gauze and boiling a solution of 1 part of 40 per cent formaldehyde to 2 parts of water under it for 30 minutes. Two pots were left as checks with no treatment. On April 15, 1925, each pot was set with a single plant of each of two selfed lines of Boone County White corn, one of which

had been self-pollinated five times and the other three times. These plants were completely freed of all seed-borne organisms by a method described in a footnote.⁶

The complete results of this test are not of interest in this paper except as they bear on the problem of soil infection. The roots of all of the plants were examined when 72 days old, with the exception that the two untreated pots and one of the pots steamed under pressure were examined at the end of 47 days. At this time the root systems of the plants grown in the untreated soil were less extensive than those of the plants grown in steamed soil, were considerably discolored, and many of the fine laterals had disappeared. Lesions were present on some of the larger roots. Many of the rootlets had a lemon-yellow cast, although otherwise appearing healthy. The roots of the plant grown in the steamed soil were pure white

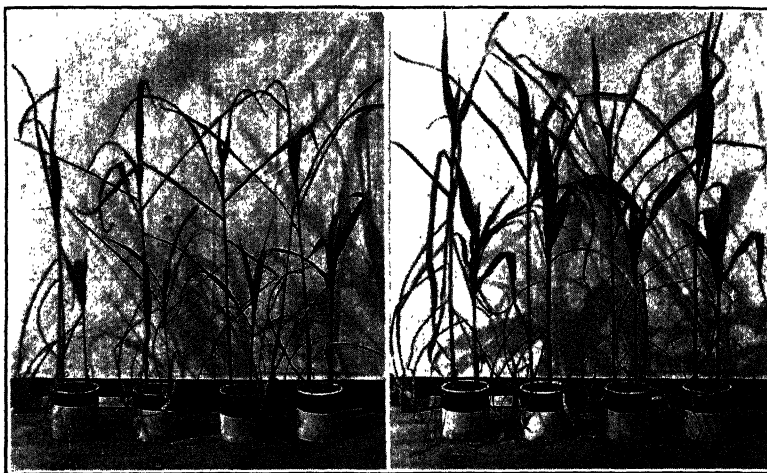


FIG. 3.—Growth of corn plants of two self-pollinated strains growing in continuous-corn soil with various heat treatments. A, soil air-dried 1 week. B, soil air-dried 2 weeks. C, soil treated at 40° to 50° C. for 1 hour. D, soil treated at 50° to 60° for 1 hour. (The plants in A, B, C, and D are comparable in size, with the exception of the small strain in pot D, which seems to show some benefit from the treatment.) E, soil heated to 100° for 1 hour. F, soil heated to 80° to 85° for 1 hour. G, soil heated to 65° to 70° for 1 hour. H, soil heated to 65° for 15 minutes. (The plants in E, F, G, and H are all larger than those in the other four pots. The root systems were white and without injury throughout, in contrast to rotting and lemon-yellow color of rootlets in A, B, C, and D)

throughout, having no rotting rootlets and none of the yellow cast mentioned. At the end of 72 days the remaining plants were of two sizes.

The plants grown in the soil that had been treated at 65° for 15 minutes and at higher temperatures were larger than those in that which had been treated at 50° to 60° or at lower temperatures or air dried (fig. 3). The reason for the difference was evident when the roots were examined. The roots of the larger plants were white and entirely without injury. In some instances the cut end of the internode where the seed was removed was not even discolored but

⁶ Briefly, the method consists in treating the seed 20 hours in a mixture of 1 part of CaO to 4 parts of water, followed by 20 minutes in a 1:1,000 mercuric chloride solution. The seeds are then germinated in culture dishes, and when the paired seminal appear they are removed together with the tap root. The seedling is then placed in a tube of nutrient agar where roots are developed from above the seed. When these roots are well developed the plant is removed from the tube, the seed cut off, and the rooted cutting planted. The entire procedure is carried out under aseptic conditions.

had healed over. Sixty-five degrees C. for 15 minutes appeared entirely effective in freeing the soil from organisms injurious to corn roots.

The root systems of plants grown in air-dry soil or soil which had been heated to 50° to 60° C. or below were less extensive, many of the small laterals having been destroyed and some of the larger roots extensively rotted, especially those on the bottom of the pot (fig. 4). The lesions on the roots in the soil that had been heated at 50° to 60° for 1 hour appeared to be more recent and less extensive than in the others of this group, suggesting delayed development of the rot organism due to heating. The injury to all of these root systems was less extensive than in the previous experiment, probably because the period of growth was shorter. Under field conditions the disease appears to act in the same way, not developing

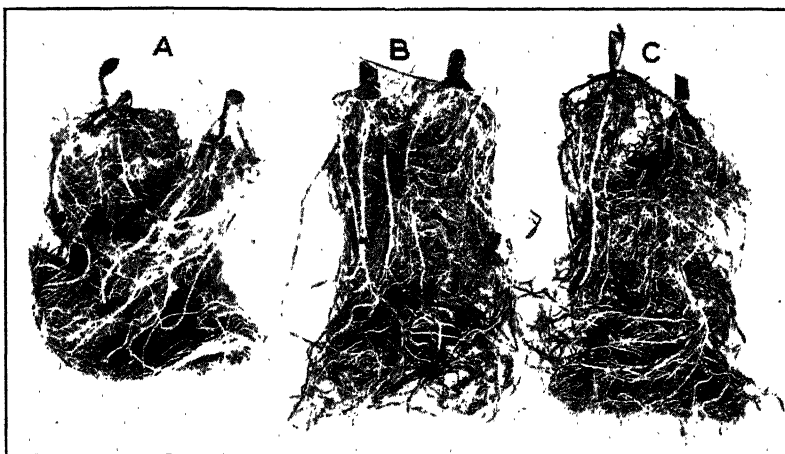


FIG. 4.—The effect of heating continuous-corn soil on the health of the root systems of corn plants. A, soil heated 65° C. for 15 minutes. B, soil heated to 50° to 60° for 1 hour. C, soil air-dried one week. The two latter pairs of plants have many large and small roots which are rotted and dark-colored, especially at the bottom of the pots; the small rootlets of these plants were often lemon-yellow. The roots of A were white and healthy throughout. Heating at 65° for 15 minutes appears to have destroyed the corn root-rot organisms, and the organism which causes the lemon-yellow color of rootlets

seriously until the plants have attained considerable growth, even in soil planted with corn continuously. There appear to be exceptions to this in which in certain areas the disease may become severe early in the life of the plant, resulting in high mortality.

Aside from the differences in rotting in one set of roots and perfectly healthy roots in the others, the two lots of roots (treated above 65° C. and below this temperature) could be separated readily on the basis of the color of the fine rootlets. The healthy roots were all pure white, while many of the rootlets of the diseased root systems were a light lemon-yellow, although appearing healthy otherwise. It is the same color which Jones (5) has described as resulting from infection with his mycorrhizal fungus. It should be noted that the roots produced in the air-dried soils had more yellow rootlets and less root rot than the two lots grown in soil treated at 40° to 50° and 50° to 60°, while within these two lots a marked difference could also be noted. The roots from the higher temperature treatment

had markedly fewer yellow roots and more rotting roots (although the rot appeared of more recent origin) than those from the soil treated at the lower temperature. This result suggests that the mycorrhizal fungus (if this is the cause of the yellowing) exerts a slight protective effect against the rot organism, especially in the smaller rootlets.

This experiment has demonstrated that a corn root rot exists which is caused entirely by organisms carried in the soil, and that in untreated soil the disease does not act as a seedling blight but causes its most severe injury later in the life of the plant.

The soil treatment with formaldehyde, although it appeared to have killed all injurious organisms, left the soil in such a toxic condition that replanting had to be repeated several times before a stand was obtained.

In a second test in which soil collected at the same time as that in the preceding test was used, and in which fungus-free corn plants were used, it was determined that in infected soil kept moist at room temperature for 94 days, both the root-rot organism and the mycorrhizal organism were reduced as compared with soil kept air-dry for this period. This relationship held in both series of pots, one set of which was examined 73 days after setting and the other 94 days after. At the first examination, roots developed in soil kept air-dry for 92 days and then exposed to intense sunlight for 14 hours had decidedly fewer yellow rootlets than either those in moist soil or in air-dry soil, while the extent of rotting was slightly less than in air-dry soil. At the time of the second examination, the roots in air-dry and air-dry and sunlight soils were about identical as to yellowing, but roots in the sunlight soil again showed less rot than those in the air-dry soil. Both showed more yellowing and rotting than in the soil kept moist.

SAND-CULTURE STUDIES

In a preliminary experiment started September 9, 1924, sand in 1-gallon glazed jars, to which a nutrient solution⁷ was added, was used in place of soil.

Plants from treated⁸ and from untreated seed were used. Two pots of treated plants were inoculated with the washings from two culture plates of a corn strain of *Fusarium moniliforme*; two were inoculated each with 30 gm. of roots from corn plants grown in a field which had been planted with corn each year for at least 9 years; two were set with contaminated seedlings, and two with treated seedlings.

The results were as follows: Contaminated seed resulted in injury to a few of the roots in the immediate neighborhood of the seed, the remainder of the roots being clean. The rootlets were clean and white on the plants from treated seed, although some rotting was evident on the temporary roots. *Fusarium moniliforme* destroyed the temporary root system but caused very little other injury than rotting of an occasional small lateral. In the pots to which corn roots were added the roots were nearly completely destroyed (fig. 5).

⁷ The nutrient solution consists of KH_2PO_4 , 1 gm.; $\text{Ca}(\text{NO}_3)_2$, 3 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 6 gm. in one liter of water.

⁸ The seed were treated by the hot-water bichloride method, germinated in steamed sand, and, when well started, removed and the seed cut off leaving the temporary roots intact. Only plants were used which appeared to have fungus-free seeds. Before setting, the plants were immersed in a weak calcium hypochlorite solution. This method is probably not efficient in freeing the plants from seed-borne fungi.

From this test and other observations it may be concluded that *Fusarium moniliforme* plays but little part in the economy of the corn plant; that it is a semiparasitic organism which appears to develop on the plant only to the extent necessary to complete its life cycle of entering the seeds, remaining there over winter, and then

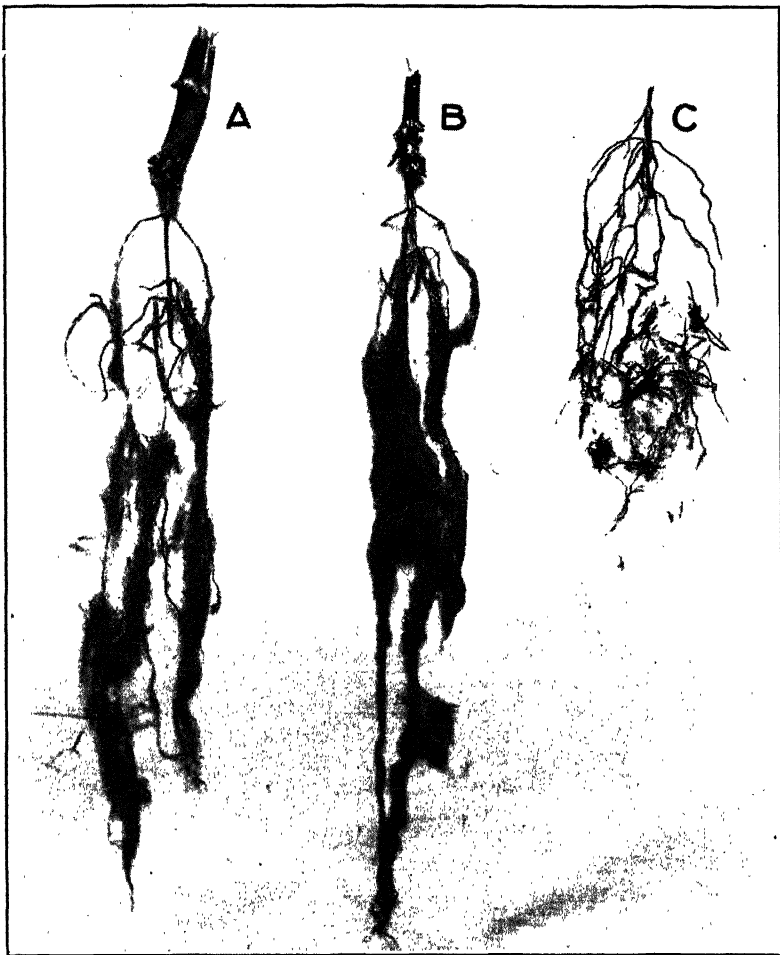


FIG. 5.—Showing effect of rotten corn roots from a continuous corn field on corn plants in sand culture. A, roots inoculated with washings from a culture dish of a pure culture of a corn strain of *Fusarium moniliforme*. B, check; no organisms added. C, sand contaminated with rotten corn roots. Treated seedlings supposedly free from seed-borne fungi were used in each case

developing to a sufficient extent on the roots so that spores will be produced so that it may again gain access to the seed; that it appears to play an important part in the destruction of the corn plant after maturity; and that organisms are present in rotten corn roots capable of causing root rot when introduced into sand cultures.

COMPARATIVE EFFECTS OF GIBBERELLA SAUBINETII AND OTHER ORGANISMS ASSOCIATED WITH CORN ROOT ROTS AND DISEASED CORN ROOTS WHEN USED AS INOCULUM

It is evident from the preceding results that a disease of corn roots may be produced in pots of either soil or sand which is similar to the disease which develops in old corn soil under field conditions, in that the injury is not a seedling blight, but develops to a serious extent after the plants have passed the seedling stage and have started to make rapid growth. Eventually, both in pot work and in the field, practically the entire root system is destroyed. It is believed that this disease is identical with corn root rot as it occurs in the field.

Several pot experiments have been conducted to determine whether this type of root rot can be produced by the organisms which are commonly supposed to cause corn root rot, namely—*Gibberella saubinetii*, *Diplodia zeae*, *Fusarium moniliforme*, *Fusarium* spp. The results of these tests may be summed up by saying that *Gibberella saubinetii* acts as a seedling blight, but if the plants recover from the initial injury, they develop in the infected medium without further trouble. Similar results have been obtained, also, with *Fusarium succisae*, which appears to be even more injurious than *G. saubinetii* to corn seedlings. Fusaria of the elegans section, and *F. moniliforme*, both isolated from rotting corn roots, do not cause the severe seedling blight caused by *G. saubinetii* and *F. succisae* but they probably cause injury to a few rootlets. They have very little or no effect on the development of the plant. The results with *Diplodia* are not conclusive, as there appears to have been an accidental contamination from pots inoculated with diseased roots. The experiments from which these conclusions were drawn will now be described.

EXPERIMENT 1

In this experiment two series of plants were grown, one in soil and the other in sand cultures. The soil used was from the experiment station farm. It had been steamed under pressure several weeks before. The cultures of interest in this paper were: (1) Steamed soil to which was added 10 gm. of sterile rice; (2) steamed soil to which were added 10 rice cultures of *Gibberella saubinetii*;⁹ (3) steamed soil plus cultures of a corn strain of *Fusarium moniliforme*; (4) steamed soil plus *F. succisae* cultures; (5) virgin soil to which were added sterile corn roots; and (6) sterile virgin soil to which were added corn roots from a continuous-corn plot where root rot was known to be present.

The plants used were of a three-year selfed line of Boone County White. The seeds for both series were treated by the lime-bichloride method, washed in sterile water, and then were transferred to tubes of nutrient agar for germination. Those which showed no signs of infection when the plant had reached the plug were then transferred, two per pot, to the soil. The plants were set January 31, 1925. In 19 days the plants in all the pots were 12 to 16 inches high. The plants in the soil inoculated with *Fusarium succisae* were at this time about two-thirds the size of the plants in the other pots.

⁹ *Fusarium succisae* was isolated from a tobacco root. It was found in test-tube cultures to be very injurious to corn seedlings, and was therefore used for comparison with *Gibberella saubinetii*, although it is probably of no significance as a corn pathogene. The *G. saubinetii* culture used was from tobacco roots. Both these organisms were identified by C. D. Sherbakoff.

Both these plants and the plants inoculated with *Gibberella saubinetii* had wilted slightly every sunny day until this time, when they both appeared to have developed a sufficiently healthy root system to supply moisture. The others had not wilted. The plants were removed at the end of 40 days. The air-dry weights of the roots and tops were as follows: Check, 48 gm.; *G. saubinetii*, 42.5 gm.; *F. moniliforme*, 41 gm.; *F. succisae*, 29 gm.; virgin soil and steamed roots, 44.5 gm.; virgin soil and diseased roots, 32.5 gm.

The original roots of the plants inoculated with *Fusarium succisae* were nearly destroyed, as were some of the first permanent roots which developed. The later roots were so nearly entirely healthy that it might be said that the plant was recovering from the initial injury. The plants inoculated with *Gibberella saubinetii* were slightly smaller than the checks and showed but slight injury to the roots. They had apparently been injured in their early development, as evidenced by the daily wilting, but had entirely recovered. The plants inoculated with *F. moniliforme* showed but an occasional injured rootlet. The roots of the check plant were healthy throughout. The roots in virgin soil with sterile corn roots added were healthy throughout, while those in virgin soil plus infected roots showed extensive injury to the laterals, many of them being completely rotted. It appeared that in but a short time the remainder would have been destroyed. In these plants the injury was evidently increasing, whereas the plants inoculated with *G. saubinetii* and *F. succisae* appeared to be recovering.

The sand-culture series of this experiment practically duplicated the soil series, except that steamed sand to which a nutrient solution was added was used in place of the soil. Inoculations were made in the same manner. The rates of growth of the plants treated in the various ways were similar to those growing in soil. The plants inoculated with *Fusarium succisae* and *Gibberella saubinetii* wilted during midday on sunny days. The plants were removed from the pots on the 25th day and the sand washed from the roots. The green weights of the plants at this time were as follows: Check, 35.1 gm.; *G. saubinetii*, 9.7 gm.; *F. moniliforme*, 29 gm.; *F. succisae*, 5.7 gm.; diseased corn roots from field mixed throughout sand, 23.3 gm.; diseased corn roots from a previous experiment placed in lower half of pot, 26.4 gm.; and same planted with an open-pollinated Boone County White plant, 60.5 gm. The roots of the check to which 10 gm. of sterile rice were added were clean throughout, except for a very few dead rootlets in the vicinity of the seed. The roots of the plants inoculated with *F. succisae* were badly rotted. The fungus was growing quite profusely around the crown of the plants. Lesions were scattered over the root system of the plant inoculated with *G. saubinetii*, but the majority of the rootlets were healthy. The injury hardly seemed sufficient to account for the slow growth of this plant, although probably more injury was caused in the seedling stage than was apparent at the time of inspection (fig. 6). The roots of the plant inoculated with *F. moniliforme* were but slightly injured, an occasional rotted rootlet being found. The three remaining pots were inoculated with diseased root material. The first, in which rotted roots collected January 17 from the field were mixed throughout the pot, had developed an extensive root system, but it was nearly completely dead and rotted when examined. In the

other two, diseased roots from a previous experiment, in which corn was grown in infected field soil, were used. The roots were mixed only in the bottom half of the pot. One of these pots was set with an open-pollinated seedling. This plant was nearly twice as large as any of the selfed plants, indicating that its early growth had been rapid and uninfluenced by the inoculum in the bottom of the pot. At the time of removal, although the sand was moist, the leaves were curled and wilted. The roots at the bottom of the pot were extensively rotted, while the rot was spreading more slowly in the rootlets higher up in the pot. The injury evidently was increasing with the age of the plant. In the other pot where the inoculum was placed in the bottom half of the pot, the roots were extensively injured in the lower half with an occasional rotted root higher up. In this series, although *G. saubinetii* and *F. succisae* both caused injury to the plants in their seedling stages, the plants in both cases appeared to recover to some extent, the later roots appearing healthy; whereas plants inoculated with diseased root material made a normal growth in the seedling stage, but developed an increasing amount of injury as the plants grew older. The two diseases can not be considered identical, the former being a seedling blight and the latter a disease of the developing plant.

EXPERIMENT 2

In this experiment 1-gallon glazed jars of steamed sand (18 pounds pressure for 2 hours) were used, to which nutrients¹⁰ were added.

The inoculum consisted of 30 grams per pot of finely cut and washed silage sterilized in 15-gram lots in culture dishes at 15 pounds pressure for 15 minutes. The dishes of silage were then inoculated respectively with pure cultures of *Gibberella saubinetii*, *Fusarium succisae*, and *F. moniliforme*. They were then incubated at 26° C. for 4 days, at the end of which time the silage was overgrown profusely with the organisms, and then added to their respective pots of sand. All were run in triplicate except *F. moniliforme*, which was used in a single pot. Thirty grams of diseased roots, collected from the field February 27, 1925, and cut into small pieces, was added to each of three pots, about a gram of the root material being put in direct contact with the roots of the plant in each case when it was set. Thirty grams of sterile washed silage was added to each of the three checks.

The plants used were a five-times selfed strain of Boone County White, prepared by the limewater-bichloride method, as in the previous experiment, and germinated in tubes of agar. Only apparently fungus-free seedlings were used. The plants were removed from the tubes with the agar adhering and set with the first node just below the surface of the sand. The plants were set March 6, 1925. Ten days later no differences could be noticed.

On March 30 the plants inoculated with diseased roots presented the best appearance; while the checks to which sterile silage had been added were next, but noticeably smaller. Two plants in one pot inoculated with *Fusarium succisae* were practically dead (seedling

¹⁰ Ten grams of finely powdered C. P. $\text{Ca}_3(\text{PO}_4)_2$ was added at the time the pots were inoculated. After the plants were set, 20 c. c. of a nutrient solution consisting of 10 gm. of KNO_3 and 25 gm. of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per 1,000 c. c. of water, was added to each pot; and also 10 c. c. of Scales' solution (25 gm. in 1,000 c. c.) was added.

blight). Two plants in one of the *Gibberella saubinetii* pots also appeared very sickly. The other plants inoculated with these organisms were all about the size of the checks, or nearly so. On April 3 the two plants in the above-mentioned *F. succisae* pot were

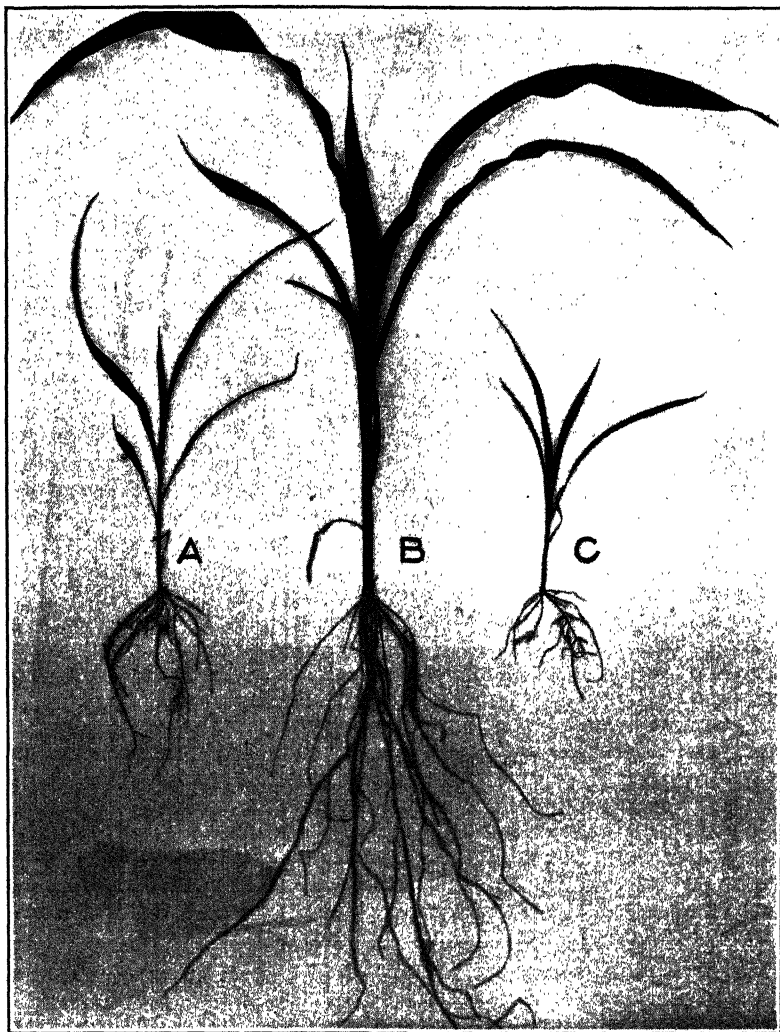


FIG. 6.—The effect of *Gibberella saubinetii* and *Fusarium succisae* on 25-day-old corn plants grown in sand cultures. A, sand, inoculated with rice cultures of *G. saubinetii*. The roots are for the most part quite healthy, although considerable injury to the rootlets probably occurred in the seedling stage. B, check; sterile rice was added. C, rice cultures of *F. succisae* added to the sand. The roots show extensive injury, although the plant appeared to be recovering

removed, as one was dead and the other nearly so. Both had developed fairly extensive root systems with a poor development of laterals, and lesions were scattered here and there on the larger roots. The death of these plants did not appear to be the result of root injury. The internode of the epicotyl and temporary roots

of both plants were rotted, and the rot had spread into the first nodes, killing the plants. On April 9 the plants were removed from one series of pots, with the exception of the *F. succisae* plants, which had already been removed. The *G. saubinetii* pot containing the two small plants was selected. The highest leaves of these plants reached 14 inches. Most of the roots were dead, on one plant only a couple of recent ones being still alive. The first internode of this plant was rotted, the injury probably extending into the first node. The second plant was somewhat larger than the first and was beginning to recover from seedling injury. The original roots and the laterals on the permanent roots were discolored, but a set of healthy roots was developing from the second node.

The plants inoculated with diseased roots were taller than the others of the series (20 inches), but were not so stocky and appeared to wilt more readily. The root systems of the plants removed were extensive and considerably injured but not so much as those inoculated with *Gibberella saubinetii*. Many of the small rootlets were yellow, although not decayed, and when sectioned were found to be penetrated by a fungus answering closely the description of Jones's mycorrhizal fungus (5).

The checks were 17 inches high when removed, a few rootlets were found to be discolored, and two larger roots had lesions. Otherwise these roots were healthy. On April 16 all of the series were going ahead of the plants infected with diseased roots. The latter were light-green and spindly, with long narrow leaves, while the others were dark green and had shorter, broader leaves.

On May 22 but little difference could be noted in the size of the plants, with the exception of those inoculated with corn roots, which were smaller (fig. 7). On May 25 the remaining plants were removed for observation of the roots.

The root systems of all plants, except those inoculated with corn roots, were about the same size and very extensive (fig. 8). In the bottom of one of the *Fusarium succisae* pots, and also in the remaining *Gibberella saubinetii* pot, a considerable number of rotting roots were present. An occasional lesion was present in the checks. The roots inoculated with *F. moniliforme* were as large as those of the check, and except for an occasional lesion they were healthy throughout. The roots of the plant inoculated with root material were nearly dead (fig. 8). The contrast in the seedling-blight injury caused by *G. saubinetii* and *F. succisae*, and root rot caused by adding infected roots, is again brought out in this test.

EXPERIMENT 3

In this experiment one-half gallon glazed jars of riversand were used, to which nutrients¹¹ were added. The inoculum added was, in all cases, root material collected April 13, 1925. It was brought to the laboratory, washed and ground in a food chopper, weighed into 30-gram lots, sterilized (when sterile roots were necessary) in culture dishes, and 15 c. c. of 5 per cent potato-dextrose solution added to each portion. Three lots were inoculated with *Gibberella saubinetii*, and three with a composite of five cultures of *Fusaria* of the *elegans* section isolated from corn roots. The cultures of root material were

¹¹ Seven grams C. P. $\text{Ca}_3(\text{PO}_4)_2$ thoroughly mixed with the sand, then 10 c. c. of the following nutrient solution was added to each pot: 100 gm. KNO_3 + 25 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per 1,000 c. c. water.

inoculated by transferring some of the mycelium of the organisms used to the tube of decoction and pouring this over the root material. The plates, including the unsterile roots plus decoction, were incubated for three days and then added to the pots of sand. Fungus-free plants (see footnote 6, p. 460) of a Boone County White strain



FIG. 7.—Eighty-day growth of corn plants in sand cultures as influenced by inoculation with *Gibberella saubinetii* (A), with rotted corn roots (B), and with sterile washed silage (C). The plants inoculated with corn roots made more rapid early growth than the others, but were soon overtaken and outgrown. If those inoculated with *G. saubinetii* pass the seedling stage without injury, they make normal growth. Seedling blight may, however, destroy them

selfed five times were then set, using two plants per pot, on April 16. On May 4 the plants in the pots inoculated with root material were severely injured. At this time plants in the three check pots appeared slightly poorer if anything than those inoculated with *G. saubinetii* and the elegans organisms, although the difference was not great. These

plants were all approximately twice the size of the root-inoculated plants. The early injury to the plants inoculated with root material in this case, as compared with the other experiments where injury was always delayed, may have been due to the fact that the causal organism was activated by incubating three days with a nutrient solution and therefore was capable of attacking the roots immediately when the plants were set. On June 12 all plants inoculated with infected corn roots were dead. On June 26 (53 days) the plants were removed from the pots and the sand washed out of the roots. Photographic records were made and the plants were air-dried. The average air-dry weights of all plants, except those inoculated with corn roots, were as follows: *G. saubinetii*, 26.3 gm.; *Fusarium spp.*, 16.5 gm.; checks, 25.4 gm.

The roots of the check plants were healthy throughout in the three series (fig. 9). The roots in two of the pots inoculated with

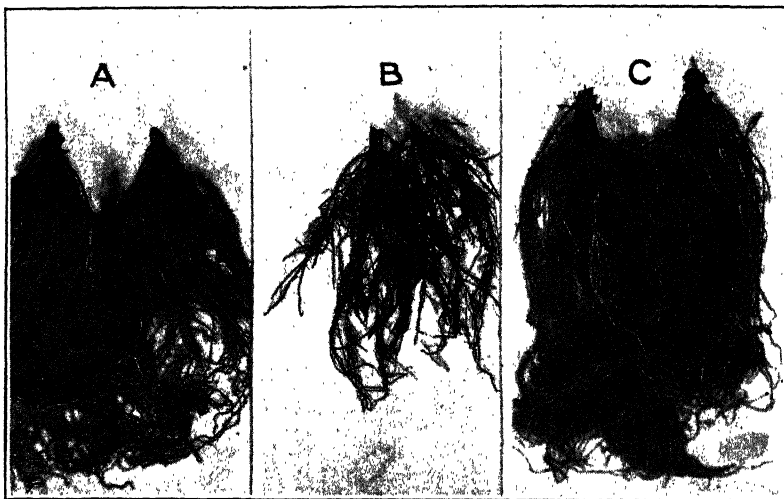


FIG. 8.—Roots of the plants A, B, and C shown in Figure 7. A (inoculated with *Gibberella saubinetii*), a few roots rotting on the bottom of the pot. B (inoculated with rotted corn roots), the roots being completely rotted. C (check), the roots appeared healthy and white throughout.

Gibberella saubinetii were practically as good as the check; while in the third the roots were very severely injured, having an appearance typical of the true corn root rot of the other experiments. The roots in two pots inoculated with *Fusarium spp.* were for the most part normal, although some of the early roots had evidently been injured, the third was in exactly the same condition as the *G. saubinetii* plant mentioned above. The roots were nearly completely rotted. As only one set of plants in both the *Fusarium spp.* series and the *G. saubinetii* series showed this condition, the writers assume that the infection was accidental from one of the root-inoculated pots. There has been evidence in other experiments that accidental contamination has occurred from the root-inoculated pots to others.

An experiment in which *Diplodia zeae* infected roots collected in the summer from diseased corn plants in the field, and a composite of 42 distinct cultures of *Fusaria*, most of which were of the elegans section, were used as inoculum was not entirely satisfactory. The

root-inoculated plants were extensively injured. Those inoculated with *Fusarium spp.* were injured on the bottom of the pot, due in part to decomposition of organic matter which left a black sludge with a distinct odor of sewage. The roots above this area, except for an occasional rotted rootlet, were healthy. The roots inoculated with *D. zeae* had grown extensively, but when removed they were found to be severely injured in both pots. Some unauthorized person had watered the plants during the progress of the experiment with a hose and caused considerable splashing, so it can not be said whether the injury was due to *D. zeae* or to accidental contamination. At least it may be said that *Gibberella saubinetii* and the *Fusaria* of the *elegans* section commonly isolated from corn roots are unable to cause corn root rot similar to that caused by rotted corn roots from a continuous-corn field.

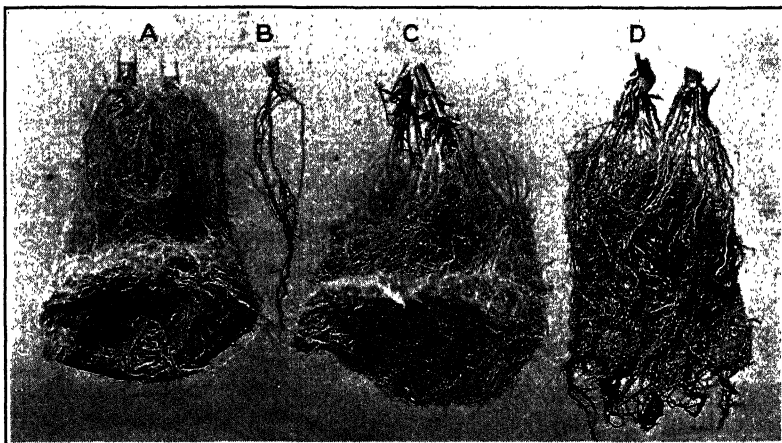


FIG. 9.—The effect of inoculation with rotted sterile corn roots (A), with rotted roots incubated with a nutrient solution for three days (B), and with rotted sterile roots plus a nutrient solution inoculated with *Gibberella saubinetii* (C). D was inoculated in the same way as C. It made rapid normal growth for a time, but gradually fell behind C. When its roots were removed D was found to have what was apparently a typical case of root rot, due probably to accidental infection by splashing from one of the root-inoculated pots. Two pots of plants in this experiment inoculated with a composite of five seemingly different cultures of *elegans Fusaria* from corn roots looked like C, while a third appeared identical with D and evidently had been infected accidentally.

RELATIONSHIP BETWEEN SOIL TEXTURE AND ROOT ROT IN POT CULTURES

In the pot experiments just reported observations were made on the distribution and spread of root rot in the various pot cultures which may throw some light on the cause of the disease. Wherever possible the complete ball of dirt or sand was removed from the jar in order to observe the roots concentrated against the inside of the pot. Rot was nearly always more severe in the bottom of the pot in the early stages of its development, but would gradually spread throughout the entire root system. In one case of evident accidental infection a streak of rotting roots was observed extending down one side of the pot, the rot evidently having followed the downflow of water. Development of rot was always more rapid in sand cultures, in which waterflow is quite rapid and where watering must be done at more frequent intervals than in the less porous soils; in fact, this

difference was so striking that an attempt was made to find a soil-sand combination in which the rot would develop about as rapidly as in sand, but would be furnished with sufficient nutrients from the soil to maintain the plant without the addition of a nutrient solution. The soil and sand combinations used were virgin soil and river sand sterilized, half virgin soil and half sand, and one-quarter virgin soil and three-quarters sand. To each of the foregoing were added rotted corn roots from a continuous-corn plot. Virgin soil plus 30 gm. of sterile roots and sand plus 30 gm. of sterile roots served as checks.

When the roots were finally examined the injury was found to be in direct proportion to the amount of sand present, the roots in soil being injured comparatively slightly, while those in sand were nearly completely destroyed. The plants were grown in 1-liter beakers so that root development and injury could be watched throughout the growth of the plant. During the progress of the experiment, when the plants were about half grown, one of the checks (sand plus sterile roots) became accidentally contaminated, evidently from one of the inoculated pots. The rot spread rapidly and when the plant was removed its roots were found to be nearly destroyed.

It is interesting to note that yellowing of the mycorrhizal type was produced both by virgin soil and by the addition of rotted roots to sterile sand, but was entirely absent in the sterile-sand-plus-sterile-roots plants.

The fact that the disease spread so much more rapidly where the flow of water was uninterrupted, suggested that the cause of the disease was probably one of the fungi which produce swarm spores or at least depended upon some free water for dissemination.

ISOLATION AND MICROSCOPIC STUDIES

Isolations have been made from some of the rapidly rotting roots from pot cultures and from plants grown in the field but have yielded usually either *Fusaria* or a fungus apparently identical with an organism commonly isolated from tobacco roots which was tentatively-identified by C. L. Shear as a species of *Hendersonia* or as *Staganospora arundinis*. *Fusarium moniliforme* was quite often isolated, but much more commonly species belonging in the section *elegans* of this genus. Tests of these organisms on corn seedlings growing in nutrient agar indicate that whereas they may cause injury under these conditions, yet the injury is of a different type from the true rot, being limited to very small laterals and to a small area of the larger roots. These tests and the pot tests reported above indicate that these organisms are probably not concerned in the problem other than as saprophytes or weak parasites which follow closely the real cause of the trouble. *Gibberella saubinetii* and *Diplodia zeae* were never isolated in these studies; and in approximately 150 separate culture plates, in each of which about five pieces of rotting roots of tobacco from several fields were planted, *G. saubinetii* was isolated but once.¹² Searches made in continuous-corn fields on the experiment station farm for the past several years by the senior author for the perfect stage of *G. saubinetii* have resulted largely in failure, especially where the field lies to the windward of all wheat fields. These results suggest strongly that *G. saubinetii* is not a common corn

¹² MILLER, P. W. [Unpublished master's thesis, Univ. Ky., Lexington, 1923.]

pathogene except as it attacks developing ears of corn, and seedlings as a result of planting seed from such infected ears, and as it attacks matured corn stalks which have been grown in the vicinity of wheat or other small grains. It does not appear to be a common soil organism, except as it is turned under on infected plant parts. It appears to have specialized largely as an above-ground organism.

Free-hand sections of the rotting edge of roots from pot cultures (experiment 3, p. 16) revealed the presence of a large-diameter, usually nonseptate fungus which is evidently a phycomycete. This has appeared consistently at the edge of the rotting tissue in the type of root injury resulting from the addition of rotted roots (fig. 10, A). Behind this zone in the rotted tissue are found oospores, often in large numbers (fig. 10, B, C). Twenty which were measured ranged from 18.8 microns to 27.0 (averaging 23.2) in diameter, including the walls.

A review of the literature on this type of root injury revealed the striking similarity between the sugar-cane root rot described by Carpenter (1) and the root rot of corn produced in either sand or soil pot cultures by the use of either diseased soil or diseased roots. (See Carpenter's plates 1 and 3.) The hyphae and the oospores in diseased roots of corn are strikingly similar in appearance to those pictured by Carpenter, and the similarity suggests a relationship between the two fungi. In a later paper Carpenter (2) showed the morphological similarity of his *Pythium*-like fungus to *Pythium Butleri* Subramaniam and *Rheosporangium Aphanodermatus* Edson. Subramaniam, as quoted by Carpenter, found his organism to be parasitic on *Nicotiana tabacum*, *Zingiber officinale*, and *Carica papaya*. In the present studies the writers have found that rotted corn roots produced a rot of tobacco similar in character to the disease produced on corn roots in pot cultures. Although it is too early to draw definite conclusions in regard to the actual cause of corn root rot, there is a strong suggestion that the Hawaiian root troubles and those of corn in the United States may be very similar in nature. The fact that the writers have not obtained a *Pythium*-like organism in isolations from rotting roots is not surprising, as Carpenter had considerable difficulty in isolating the organism until a favorable medium was discovered.¹³ In 1919 Clinton (3, p. 428-430) obtained plants of corn severely affected with root rot, and found, in the pith of the stubble in the vicinity of the nodes, the oospores of a fungus which he identified as *Phytophthora cactorum*, but he was not certain whether it was a *Pythium* or a *Phytophthora*. The oospore measurements and appearance of the spores correspond closely with those illustrated in this paper.

CONCLUSIONS

A series of experiments have been conducted using both soil and sand in an attempt to differentiate between the types of injury caused by the more or less commonly accepted corn root rot organisms. It has been determined that through the use of soil from a continuous-

¹³ Since the completion of this work, one of the writers (Karraker) was informed by James Dickson, at the November, 1925, meeting of the American Society of Agronomy in Chicago, that a *Pythium*-like fungus had been isolated from corn roots in his laboratory; and more recently still, the program of the seventeenth annual meeting of the American Phytopathological Society announces a paper by Helen Johann, J. R. Holbert, and James G. Dickson on "A *Pythium* seedling blight and root rot of dent corn."



FIG. 10.—A, Pythiumlike fungus near the border of rotting and healthy tissue of a corn rootlet grown in sand culture to which dead corn roots from a continuous-corn field were added. This type of organism has been found consistently in the rotting rootlets examined. Penetration of the host cell wall may be seen in the rotting rootlets, $\times 1000$. B, C, Pythiumlike oospores consistently present in the rotting rootlets examined in the decayed zone immediately behind the type of fungus shown in A. These spores are usually abundant in rotted tissue in sand cultures. B, free-hand section; and C, crushed rootlet $\times 1000$.

corn plot, in the absence of seed-borne organisms, a severe rotting of corn roots may be produced. The addition of rotten roots collected from a continuous-corn field in the fall, midwinter, and spring, and added to sand cultures in which fungus-free corn plants were grown, resulted in a severe root rot. Rotting was found to progress more rapidly in sand where there was a free movement of water than in soil. The disease did not act as a seedling blight in pot cultures unless the causal organism was previously activated by incubation of the rotted roots with a nutrient solution.

Both *Gibberella saubinetii* and *Fusarium succisae* have been found to cause seedling blight, but if plants escape seedling injury, they appear to make as rapid growth as check plants. In more than 100 isolations made from rotting corn roots from plants growing in the field, in pot cultures of diseased soil, or in sand cultures inoculated with rotten corn roots, these organisms have not been obtained in a single instance. *Diplodia zeae* has not been obtained in these isolation studies. *F. moniliforme* and species of *Fusarium* of the *elegans* section are commonly isolated from rotting corn roots. *F. moniliforme* appears to rot only an occasional root in sand cultures, and a composite of 42 cultures of *Fusaria* from corn roots, most of which were of the *elegans* section, caused but slight injury. Apparently they are concerned in the problem only as secondary invading organisms.

Free-hand sections of rotting corn roots grown in infected soil, or in sand cultures inoculated with rotten corn roots, have consistently revealed a large-diameter, generally nonseptate organism, and numerous oospores of the *Pythium* type. The type of injury produced in soil and sand cultures is remarkably similar to the injury (pictured by Carpenter) to Lahaina cane grown in sick soil and caused by a *Pythium*-like fungus. The suggestion is made that corn root rot, other than seedling blights known to be caused by certain seed-borne organisms, is caused by a fungus similar to the fungus causing the cane root rot.

Rotted corn roots added to sand cultures of tobacco are capable of causing a type of injury which appears identical with the so-called brown root rot of tobacco in the field.

The roots of corn plants grown in continuous-corn soil, in virgin forest soil, or in sand to which rotted corn roots from the field have been added, develop lemon-yellow discolorations; and on sectioning the roots are found to be penetrated by a fungus similar to the mycorrhizal fungus described by Jones. This organism and the corn root-rot organism are both destroyed by maintaining the soil at 65° C. for 15 minutes.

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A BLOSSOM AND SPUR BLIGHT OF PEAR CAUSED BY A STRAIN OF BOTRYTIS CINEREA PERS.¹

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INTRODUCTION

As far as the writer is aware, this paper is the first report in the literature of Botrytis having been found to be the cause of a blossom blight of pear, *Pyrus communis*. This blight of *Pyrus communis*, caused by a strain of *Botrytis cinerea* Pers., was first reported by the writer² in 1920, at that time as a disease of the spur. In April and May of that year specimens of blighted spurs were sent to the Oregon Agricultural Experiment Station from two localities in Douglas County, Ore. In one orchard in one of those localities from 10 to 15 per cent of the fruiting spurs of some Anjou pear trees were infected, and somewhat less infection was found in Winter Nelis

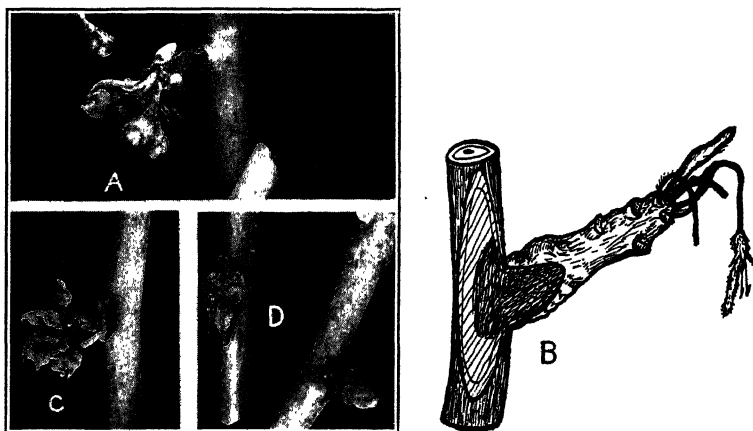


FIG. 1.—A, Diseased spur of Anjou pear that was attacked by Botrytis “in the pink” stage of the buds. When this photograph was taken the invasion had extended into the parent branch; B, diagrammatic illustration to show how far the mycelial invasion and discoloration progressed into the parent branch in one instance; C, photograph of a diseased spur taken the same spring that infection took place. Note the cankerous condition at the base of the spur; D, photograph showing canker at the juncture of the spur with the parent branch

and Comice trees. Since 1920 the disease has been found mainly on Anjou stock, but Bartlett, Nelis, Comice, and Bosc have been slightly affected in the order mentioned.

DESCRIPTION OF THE DISEASE

Infection takes place through the flower parts. The first visible evidence of the disease is the wilting of one or more flowers or flower

¹ Received for publication Jan. 20, 1926; issued September, 1926.

² ZELLER, S. M. A SPUR BLIGHT OF PEAR CAUSED BY BOTRYTIS. (Abstract) *Phytopathology* 11: 105. 921.

buds of a spur. If only one flower is affected, the mycelium makes its way down the flower pedicel in one or two days. When the infection reaches the base of the pedicel it spreads to the bases of other flower pedicels, and the flowers which these pedicels support quickly wilt, and the whole spur has a diseased appearance (fig. 1, A). As is always true in the case of pear tissues, the affected parts quickly darken. Infection and discoloration continue rapidly down the spur and advance for some distance into the parent branch (fig. 1, B). This whole invasion takes place within two to four days, so there may be many thoroughly diseased spurs before the petals fall from healthy flowers. Fortunately, infection takes place at a time of vigorous growth of the parent branch. The vigorously growing pericycle producing a cork cambium about the diseased area at the base of the spur serves as an expeditious check against the further advance



FIG. 2.—Photographs of the vegetative fruits produced just above cankers that are caused by *Botrytis* on Anjou stems or petioles. In A, the canker has forced the fruit away from the cortex of the stem. In B and C the fruit is an enlargement of the petioles

of the mycelium. As soon as the spread of the mycelium is checked the base of the diseased spur becomes cankerous in appearance, owing to the heaving and splitting of the margins of the dead bark when callus begins to form (fig. 1, C). In a short time the flower parts fall away, and in late spring and early summer the diseased spurs appear, as illustrated in Figure 1, D. If the spurs are taken during these stages of the disease, the causal organism may be isolated from the diseased tissues.

Although such cases are seldom found, infection may occur after the fruit has set. In such a case infection starts through the sepals, but the invasion progresses more slowly than described above. The invasion slowly spreads in the cortex of the immature fruit, which usually drops before it is 8 to 10 mm. in diameter. In cases of fruit infection the fungus seldom gets beyond the suberized layer of the normal abscission layer at the base of the pedicel. When the in-

vasion does exceed this limit the small area of diseased tissues below the abscission layer is limited, being occluded from the vigorously growing healthy tissues by a rapidly formed phellogen layer. These single late-infected fruits or flowers dry and fall away late in the spring or early summer. Collateral products of the small cankers on the parent branch are the vegetatively produced false fruits. Parthenocarp in Anjou pears when floral parts are present has been illustrated and discussed by Kraus and Kraybill.³ When *Botrytis* canker occurs in such a position on a small twig as to cut off the downward translocation of food substances from leaves above, these vegetative pears may be found (as illustrated in fig. 2, A, B, C) immediately above the canker as a fleshy enlargement of the outer cortex of the stem or of the petioles of the leaves furnishing the nourishment

DISSEMINATION OF THE DISEASE

The *Botrytis* organism remains dormant during the summer in the diseased spurs and cankers. In the fall, after the rains begin, sclerotia are formed in the crevices and on the surfaces of the diseased tissues. The following spring the sclerotia are covered with conidiophores (fig. 3), which shed clouds of conidia upon agitation of the spurs. Since the hold over of the disease is in the spurs which were infected the preceding season, as well as in other plant debris, control by the elimination of such plant debris would be hopeless.

PROOF OF PATHOGENICITY

The pathogenicity of this strain of *Botrytis cinerea* has been established by the constant association it has with the type of injury described above, and by inoculations made with conidia from pure cultures. The disease has been collected many times in the Umpqua Valley in Oregon, and has been sent to the Oregon Agricultural Experiment Station from several localities in that district and also from the Grants Pass and Medford districts of the Rogue River Valley. In 1923 it was reported from Antelope Valley and similar localities in California.⁴ The causal organism has been isolated from samples of all of the cases reported in Oregon.

Inoculations were made in three different ways: (1) Spores were dusted on the flower parts; (2) spores were sprayed on in a water suspension; and (3) a water suspension of spores was injected into

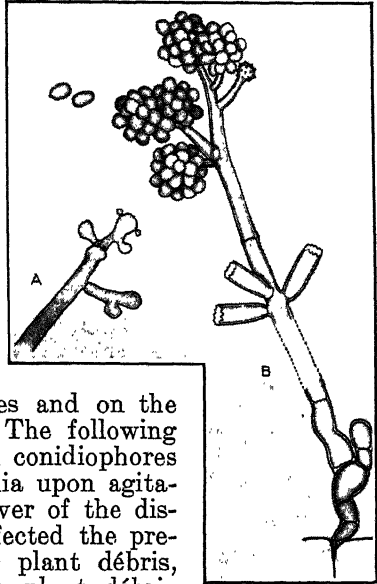


FIG. 3.—Conidiophores and spores of the strain of *Botrytis cinerea* Pers. which causes blossom and spur blight of pear. A, mature conidiophore. $\times 280$. B, immature conidiophore, with spores beginning to form

³ KRAUS, E. J., and KRAYBILL, H. R. VEGETATION AND REPRODUCTION WITH SPECIAL REFERENCE TO THE TOMATO. Oreg. Agr. Expt. Sta. Bul. 149, 90 p., illus. 1918.

⁴ SMITH, E. H. MINOR APPLE AND PEAR DISEASES. Calif. Agr. Expt. Sta. Rpt. 1922/23: 185. 1923.

the bark of spurs with a hypodermic needle. In the cases where the latter method was used the spore dilutions in sterile water were injected into 20 spurs each of Anjou and Bartlett pears on March 23, 1921. Nineteen (or 95 per cent) of the Anjou spurs were killed in 6 to 12 days, while 16 (or 80 per cent) of the Bartlett spurs were withered in the same period. When spores from a pure culture which was obtained from wilted Anjou flowers were dusted on Anjou blossoms in the greenhouse, the blossoms being in all stages from "in the pink" to full bloom, 20 spurs showed wilted blossoms in 4 days while 20 spurs which were not dusted with spores were normal. Infection was accomplished as successfully when the spores were sprayed on flowers as when they were dusted on. The dusting inoculations were repeated under field conditions. Out of 30 Anjou spurs inoculated with dry conidia 26 showed one or more blossoms wilted in five days. The relation of humidity and temperature to infection has not been studied.

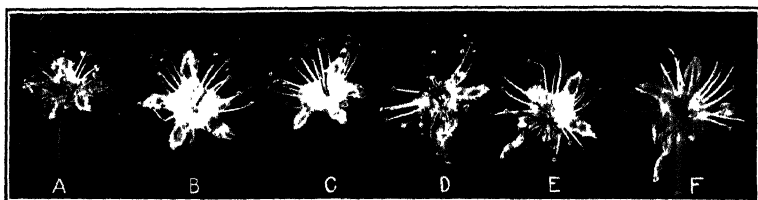


FIG. 4.—Pear flowers, with the petals plucked away so as to show the various courts of infection. A, about half of the flower infected; B, one style infected; C, two styles infected; D, all of the styles infected and the infection is advancing into the calyx cup from two sides; E, infection from a petal has nearly reached the pistils. Two styles, seen to be partially discolored, were infected later; F, petal infection has extended into the calyx

INCIPIENCY OF INFECTION

Ward,⁵ in his classic study of a lily disease caused by *Botrytis cinerea*, demonstrated that infection was possible through extracellular enzyme activity which digested the superficial walls of the host tissues. On the other hand, Blackman and Welsford⁶ observed that the germ tube of *B. cinerea* on the surface of the leaf of broad bean (*Vicia faba*) excreted a mucilaginous sheath by which the tube was firmly cemented to the surface. A depression in the epidermal wall below the germ tube suggested that considerable pressure was exerted on the substratum. Actual penetration of cuticle was by means of a peglike growth that was developed from the underside of the germ tube which was in close contact with the cuticle, and thus penetration could occur without there being an appressorium. No influence of enzyme action on the cuticular walls could be detected, but such activity was evident only after the mycelium had penetrated to the parenchyma of the leaf. Death of the epidermal cells prior to the penetration of the cuticle was never found to occur.

In the case of the strain of *Botrytis cinerea* causing blossom and spur blight of pear, the stages of infection observed were rather distinct and worthy of mention here. When conidia were dusted on

⁵ WARD, H. M. A LILY DISEASE. Ann. Bot. [London] 2: 319-332, illus. 1888.

⁶ BLACKMAN, V. H., and WELSFORD, E. J. STUDIES IN THE PHYSIOLOGY OF PARASITISM. II. INFECTION BY *BOTRYTIS CINEREA*. Ann. Bot. [London] 30: 389-398, illus. 9116.

pear blossoms in the greenhouse, infection started in one of four places. In some flowers the stamens wilted first, with the infection apparently having its incipency somewhere along the filament; invasion proceeded from the filament to the receptacle cup and thence to the neighboring petals, sepals, stamens, and styles, causing immediate browning and wilting. In other flowers styles were infected first, whereas in still others sepals were first infected. In a very few cases small bracts on the flower pedicels became infected first.

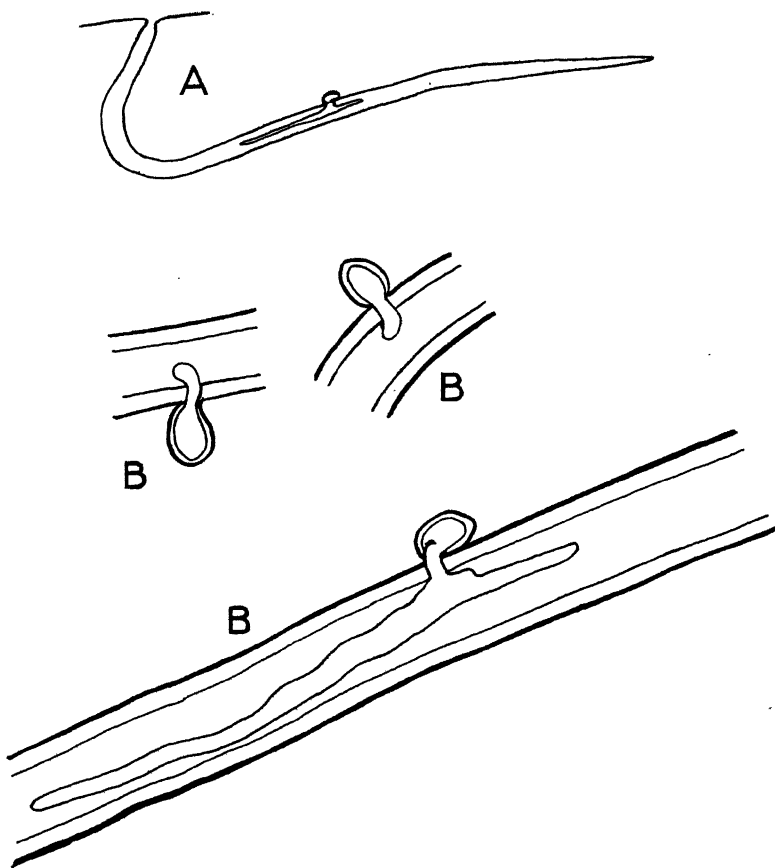


FIG. 5.—A, epidermal hair from the filament of a stamen of a pear blossom, showing the spore and germ tube after infection has taken place. $\times 115$. B, camera-lucida diagrams of the spores and germ tubes in the incipient stage of infection of the epidermal hairs of stamens. $\times 720$

Figure 4 shows several flowers in the early stages of blight. Figure 4, A, shows about one-half of the flower invaded. In Figure 4, B, the petals have been plucked away to show the single infected style. In Figure 4, C, two styles are attacked. Figure 4, D, shows two sides of the receptacle and all of the styles infected. Figure 4, E, shows two styles and one side of the cup infected. Figure 4, F, is an example of petal infection which has invaded a short distance into the receptacle.

Mycelial invasion of the tissues proceeds very rapidly. For instance, a stamen was found somewhat browned and beginning to wilt at 9 a. m. At 2.15 p. m. the invasion had spread until about one-fourth of the calyx disk was browned and one petal and two sepals were wilted.

Microscopic examination revealed that infection may take place by the conidia adhering to the epidermal hairs of the floral parts and the germ tubes entering directly through the wall of the hairs. This was observed in many cases, and no instance of mechanical pressure or peglike growth from a superficial germ tube was observed. After penetrating the wall of the hair the germ tube makes rapid growth toward the epidermal cell. Figure 5, A, shows a hair from the stamen of an Anjou flower. Figure 5, B, shows several early infections of hairs greatly enlarged.

There has been no opportunity for trials of sprays. The disease has not been continuously severe enough to warrant the growers to expend time and materials for sprays to control this disease alone.

SUMMARY

A spur and blossom blight of pears in Oregon is described. It attacks the Anjou, Bartlett, Winter Nelis, Comice, and Bosc in severity in the order mentioned. As far as the writer knows the disease is limited almost entirely to southwestern Oregon. As many as 15 per cent of fruiting spurs have been killed in the most severe cases found affecting Anjou pear. Conidia from sclerotia on hold-over lesions infect through the hairs of the floral parts.

THE RELATIVE UTILIZATION OF FEED ENERGY FOR MAINTENANCE, BODY INCREASE, AND MILK PRODUCTION OF CATTLE¹

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INTRODUCTION

In 1924 a paper² representing cooperative work by the Institute of Animal Nutrition of Pennsylvania State College and the United States Department of Agriculture was published, in which preliminary results on the relative utilization of energy in milk production and body increase of dairy cows were given. Further data of a generally similar but greatly improved character are now available, and these are given in the present report. In the earlier study the heat production and gains of energy were computed from the carbon and nitrogen balances, the maintenance requirement of energy was estimated from published averages, the net energy utilized for body gain was estimated from results with a single cow, and the computed heat production was not corrected to a standard day, as to standing and lying.

In the experiments upon which the present report is based several cows were used, the heat production and gains of energy were determined by direct calorimetry, the maintenance requirement of net energy was also directly observed with each cow, as the fasting katabolism, the net energy utilized for body gain was determined with each cow while she was dry, and a new procedure was utilized for computing the heat production to the standard day as to standing and lying.

The investigation herein reported comprises three series of experiments,³ conducted during the years 1921, 1922, 1923; and it deals with the efficiency of the milk cow, in terms of energy, to utilize feeding stuffs, especially for milk production, in comparison with maintenance and body gain.

PLAN OF THE INVESTIGATION

The utilization of feed energy in milk production is here considered in relation to that portion of the feed which is available for milk production after deducting the nutritive requirements of maintenance and any such body gain as may have occurred. The milk energy is thus regarded as net energy utilized in the production of milk.

The schedule of the experiments involving milk production is set forth in Table 1. The first two experiments, 221E and 221F, were planned to cover two series of observations with each cow, one as early and one as late as practicable in the period of lactation, and on a plane of nutrition estimated to be slightly above the requirements

¹ Received for publication Apr. 1, 1926; issued September, 1926.

² FRIES, J. A., BRAMAN, W. W., and COCHRANE, D. C. RELATIVE UTILIZATION OF ENERGY IN MILK PRODUCTION AND BODY INCREASE OF DAIRY COWS. U. S. Dept. Agr. Bul. 1281, 36 pp., illus. 1924.

³ Of these the first two were planned by the late H. P. Armsby, the third by J. A. Fries; and all were conducted under the immediate supervision of J. A. Fries, with the cooperation of W. W. Braman, D. C. Cochran, Max Kriss, C. D. Jeffries, W. J. Sweeney, and R. M. Meredith.

for maintenance and milk production, but not permitting of fattening to a significant extent. The third experiment, 221G, was planned to determine, in conjunction with the study of utilization of feed energy, the effects upon milk production of different quantities of the same feed. This last experiment consisted of four periods with one cow on widely different quantities of the standard feed mixture. During the first and fourth periods the cow was given as much feed as she would eat without waste; in the second period the ration was as computed to equal the maintenance requirement; and in the third period the ration was increased by half of the difference between those of the first and the second periods.

TABLE 1.—*Experimental periods, rations, and live weights of experimental subjects*

Experiment No.	Cow No.	Period No.	Preliminary feeding period	Collection period	Rations		Feed refused (average per day)	Average live weights
					Alfalfa hay	Grain		
221E	874	I	Dec. 25-Jan. 4	Jan. 5-Jan. 14, 1921	<i>Kgm.</i> 3. 140	<i>Kgm.</i> 4. 700	<i>Kgm.</i> None.	<i>Kgm.^a</i> 381
	874	II	Mar. 12-Mar. 22	Mar. 23-Apr. 1, 1921	3. 040	4. 560	None.	391
	886	I	Jan. 8-Jan. 18	Jan. 19-Jan. 28, 1921	3. 700	5. 540	None.	398
	886	II	Mar. 26-Apr. 5	Apr. 6-Apr. 15, 1921	3. 200	4. 800	None.	401
221F	886	I	Dec. 23-Jan. 3	Jan. 4-Jan. 13, 1922	4. 370	6. 560	^b 0. 774	416
	886	II	Mar. 25-Apr. 4	Apr. 5-Apr. 14, 1922	3. 660	5. 490	None.	412
221G	887	I	Nov. 25-Dec. 5	Dec. 6-Dec. 15, 1922	3. 651	5. 476	None.	383
	887	II	Dec. 23-Jan. 2	Jan. 3-Jan. 12, 1923	1. 750	2. 620	None.	346
	887	III	Jan. 13-Jan. 23	Jan. 24-Feb. 2, 1923	2. 700	4. 050	None.	345
	887	IV	Feb. 3-Feb. 13	Feb. 14-Feb. 23, 1923	3. 650	5. 480	None.	358

^a Average of eight daily weighings, taken before watering, at about 8 a. m., during the collection period.

^b Average of 10 days. Of the entire amount refused, 3.31 kgm. were refused during the two days in which the calorimetric study was made.

Each experimental period covered 21 days, the first 11 being preliminary, and the last 10 the collection period. During the latter period the milk, urine, and feces were collected and sampled for chemical analysis; and during the last two days of each collection period the animal was subjected to a respiration calorimetric study in which heat production, carbon dioxide, water vapor, and methane were measured.

EXPERIMENTAL SUBJECTS

Three Jersey cows were used in these investigations. Two of them, Nos. 886 and 887, were purebred, and one, No. 874, was of unknown breeding. Cow 886 was born July 13, 1914. Her first calf was dropped October 10, 1917, her second calf November 28, 1920, and her last calf December 24, 1921. She was used dry in experiment 221D (1920), and fresh in experiment 221E and 221F (1921 and 1922). Cow 874 was born May 5, 1916. Her second calf was dropped July 27, 1920. She was used fresh in experiment 221E, and dry in experiment 221F. Cow 887 was born September 5, 1917. Her third calf was dropped October 27, 1922. She was used dry in experiment 221F, and in milk in experiment 221G (1923). These animals possessed the usual nervous temperament of Jersey cattle. No one was a hearty feeder, and all were rather slow in adapting themselves to the changes in ration. All were fair milkers, for their breed.

FEEDS AND RATIONS

The standard ration used throughout all experiments considered in this paper consisted of alfalfa hay of good quality cut into about 1-inch lengths, and a grain mixture composed of 10 per cent linseed meal, 30 per cent wheat bran, 30 per cent ground oats, and 30 per cent corn (maize) meal, all of good quality. The ratio of hay to grain as fed throughout all the experiments was as 2 to 3, by weight. Common salt was fed in the amount of 30 gm. per day, 15 gm. at each feeding.

In consideration of the nature of the ration, and of its individual components, it was assumed that the amino acids of the protein mixture were in efficient variety, and that the mineral and vitamin requirements of the animals were satisfied.

On account of the disqualifying refusal of feed by cow No. 886, in Period I of experiment 221F, and other technical difficulties with cow No. 874, in Period II, experiment 221E, the data from these periods are excluded from this discussion.

METHODS OF EXPERIMENTATION

The rations were weighed in covered tin cans in which they were kept until needed; and the sampling was performed by a carefully considered procedure which need not be described in detail.

The urine and feces were collected together. At the end of each 24 hours these excreta were weighed, thoroughly mixed, and an aliquot weighed out for a composite sample. Another portion was weighed in a shallow tin pan, and dried at about 60° C. The composite sample was preserved in a zinc can by the addition of carbon bisulphide, and at the end of the 10-day collection period the composite sample was well mixed, and a portion dried and prepared for analysis, as were also the daily samples.

Samples of the milk were preserved by the addition of 1 c. c. of 40 per cent formalin per liter. From these daily samples aliquots were weighed out for the composites. These composite samples were preserved, in duplicate, and kept cool until the analyses were completed.

The milking was done at half past 4 o'clock, morning and evening. For this purpose the main door of the calorimeter was opened, thus permitting the milker to enter. Corrections for the heat, carbon dioxide, and water vapor given off by the milker while in the calorimeter were made on the basis of published data considered satisfactory for the purpose.

The feeds and dried excreta were analyzed by methods conforming in all essential details to the procedures of the Association of Official Agricultural Chemists;⁴ the heat of combustion and the carbon being determined by means of a bomb calorimeter; and in the last two experiments carbon was also determined by means of a combustion furnace. Nitrogen was determined in the fresh excreta by the König method in all experiments.

⁴ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

Experiment 221G, Cow 887, Period I				Experiment 221G, Cow 887, Period II							
Income:	Alfalfa hay.....	3,241.5	418.0	77.4	1,495.0	14,680.4	1,553.7	186.0	37.1	710.6	7,036.5
	Mixed grain.....	4,791.7	668.0	122.1	2,231.2	22,530.5	2,292.6	318.0	58.4	1,067.5	10,776.8
	Water.....		17,000.0					11,100.0			
	Total.....	8,033.2	18,146.0	199.5	3,726.2	37,210.9	3,846.3	11,604.0	95.5	1,784.1	17,816.3
Outgo:	Feces and urine.....	a 3,223.4	11,223.0	b 147.0	b 1,378.6	a 13,873.1	a 1,734.5	6,818.7	b 93.1	b 718.8	a c 7,054.6
	Methane.....	198.7			148.7	2,651.5	104.0			77.9	7,387.8
	Milk.....	1,347.0	7,456.2	43.6	705.4	7,947.2	891.5	5,162.6	27.5	482.6	5,588.5
	Hair and snuff.....	10.6	0.4	0.9	4.8	53.2	6.8	0.3	0.6	3.1	33.8
	Carbon dioxide.....	5,452.6			1,486.9		3,450.7			941.0	
	Water vapor.....		5,951.9					3,141.1			
Metabolizable:	Income minus urine, feces, and methane.....					20,886.3					9,373.9
Body balances:	Fat.....	-10.06			-7.7	-95.6	-408.3			-358.3	-4,448.9
	Protein.....	+18.0		+3.0	+9.5	+80.2	-154.2			-81.0	-887.4
	Water.....		-6,485.5						-25.7		-887.9
	Computed heat production.....					12,701.3		-3,518.7			
Experiment 221G, Cow 887, Period III				Experiment 221G, Cow 887, Period IV							
Income:	Alfalfa hay.....	2,383.4	296.0	60.4	1,092.3	10,759.2	3,222.0	448.0	81.6	1,476.6	14,544.8
	Mixed grain.....	3,551.2	498.0	91.4	1,656.9	16,719.4	4,805.1	449.0	123.7	2,242.0	22,622.8
	Water.....		15,725.0					19,735.0			
	Total.....	5,934.6	16,519.0	151.8	2,749.2	27,478.6	8,027.1	20,632.0	205.3	3,718.6	37,167.6
Outgo:	Feces and urine.....	a 2,402.1	10,452.9	b 110.3	b 1,014.3	a 10,198.3	a 3,327.0	14,317.7	b 153.3	b 1,408.8	a c 14,276.6
	Methane.....	153.7			115.0	2,691.0	194.5			145.6	2,595.4
	Milk.....	951.8	5,945.3	33.4	505.2	5,945.3	1,049.1	6,308.8	40.5	593.0	6,044.5
	Hair and snuff.....	7.0	0.3	0.6	3.2	35.0	9.9	0.9	0.9	4.5	49.5
	Carbon dioxide.....	4,309.4			1,175.2		5,242.8			1,429.7	
	Water vapor.....		4,200.0					6,386.4			
Metabolizable:	Income minus urine, feces, and methane.....					15,229.3					20,205.6
Body balances:	Fat.....	-114.1			-87.3	-1,084.0	+200.8			+153.6	+1,907.6
	Protein.....	+45.0		+7.5	+23.6	+200.6	+63.6		+10.6	+33.4	+283.5
	Water.....		-4,079.5					-6,331.8			12,010.5
	Computed heat production.....					10,429.9					

Corrected for loss on drying

Corrected to nitrogen equilibrium

Determined on fresh substance

* Corrected to nitrogen equilibrium.

b Determined on fresh substance.

c Corrected for loss on drying

In the air current entering the calorimeter the carbon dioxide and water vapor were determined gravimetrically in a continuous sample, and in the outcoming air the carbon dioxide, water vapor, and methane were likewise determined in a continuous sample. Determinations were also made of oxygen and of carbon dioxide, in the outcoming air current, by means of a Sonden apparatus, on samples collected at half-hour intervals during the last 24 hours of each calorimeter period.

THE BALANCE OF MATTER AND ENERGY

The income, outgo, and balances of matter and energy for each cow and for each experimental period are recorded in Table 2. These foundation data are submitted for record only. The balance of energy was computed from the balances of protein and fat, by the use of the usual factors, the energy of the protein being corrected to nitrogen equilibrium, as was also the energy of the feces-and-urine mixture. It should be borne in mind that these energy balances rest upon a different experimental basis from those appearing in Table 3, which were obtained by the use of the directly observed heat production. This direct heat production and the computed heat production do not differ in any case to an important degree. The significance of these data will be discussed in the later, derived tables.

TABLE 3.—*Energy of milk produced and of body gain, based on the observed heat production*

Experiment No.	Cow No.	Period No.	Dry matter in feed eaten	Metabolizable energy		Observed heat production	Energy of milk	Gain of energy by body
				Per kgm. of dry matter of feed	Total			
			<i>Kgm.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221E	874	I	6.879	2,462	16,934	11,417	4,931	+586
	886	I	8.105	2,479	20,090	12,048	6,498	+1,544
		II	6.990	2,572	17,978	11,049	5,102	+1,767
221F	886	II	8.067	2,571	20,741	12,422	5,935	+2,384
221G	887	I	8.033	2,575	20,080	12,308	7,047	+371
		II	3.846	2,437	9,374	8,627	5,589	-4,842
		III	5.935	2,506	15,229	9,870	5,648	-280
		IV	8.027	2,528	20,296	11,670	6,045	+2,581

ENERGY OF MILK PRODUCED AND OF BODY GAIN

The metabolizable energy (gross energy minus energy of excreta) per kilogram of dry matter of feed (Table 3) agreed satisfactorily for the different cows and periods, indicating the existence of consistent experimental conditions. To explain the significance of this table, it should be stated that the total metabolizable energy, minus the observed heat production and the energy of the milk, equals the gain of energy by the body.

The observed heat production, the energy of the milk, and the energy of the body gain differed, from period to period, as determined by the differences in plane of nutrition. Since the cow tends strongly to maintain her individually characteristic milk production, regardless of variations in the feed, the radical changes in plane of nutrition affected the energy of the body gain much more prominently than they did the energy of the milk.

Attention is called to the fact that the observed heat production as recorded in Table 3 has not been corrected to the standard day, as to standing and lying; hence the balances of energy cover the individuality of the cows with regard to time and energy spent in these two positions.

Before the net-energy values of the feed for milk production can be computed it must first be known how much feed and metabolizable energy should be allowed for the maintenance of these animals, and for any gain of energy by the body which occurred.

The computation of these requirements (of feed and metabolizable energy) was based upon (1) the maintenance requirement of energy (heat production of fasting), (2) the net-energy value of the ration, and (3) the percentage utilization of the metabolizable energy of the ration for maintenance, the derivation of which data is as indicated in Table 4.

TABLE 4.—*Computation of the net-energy value of the ration and the utilization of the metabolizable energy in the maintenance of dry cows*

Experiment No.	Cow No.	Period No.	Body surface in maintenance periods	Fast-ing katabolism per square meter per day ^a	Total fasting katabolism	Heat pro-duction in main-tenance periods per day ^a	Heat incre-ment		Metab-oliza-ble energy per kilo-gram dry matter	Net energy per kilo-gram dry matter of feed	Utiliza-tion of metab oliza-ble energy
							Total	Per kilo-gram dry matter of feed			
221D-----	886	I III	Sq. m. 4.70 4.84	Cal. b1,331 b1,331	Cal. 6,256 6,442	Cal. 8,085 8,028	Cal. 1,829 1,584	Cal. 480 419	Cal. 2,429 2,476	Cal. 1,949 2,057	Per cent 80.2 83.1
Average-----					6,349			450	2,453	2,003	81.7
221F-----	874	II	4.81	c1,446	6,955	9,093	2,138	534	2,397	1,803	77.7
	887	II	4.08	c1,483	6,051	8,274	2,223	613	2,484	1,871	75.3

^a Twelve hours standing and 12 hours lying.
^b Results of the third day of fast.
^c Average of days of fast 6½ to 7½ and 7½ to 8½.

The fasting katabolism of cow 886 (fifth column of Table 4) is for the third day of fast, whereas in the case of cows 874 and 887 the data represent the average of days 6½ to 7½ and 7½ to 8½ of fast. It will be noted that the total fasting katabolism per day has been computed according to the estimated body surface of the animals in the maintenance periods, so as to render the metabolism in the fasting and maintenance periods comparable in respect to surface area.

It should be understood that the methods followed in this computation are provisional only, and that the determination of the fasting katabolism as a measure of the maintenance requirement of energy has yet to be standardized.

The difference between the heat produced per day in the maintenance periods and the heat produced per day while fasting is the heat increment, or energy cost of feed utilization. Subtracting the heat increment per kilogram of dry matter of the ration from the metabolizable energy per kilogram of dry matter in the maintenance periods gives the net energy value of the feed, per kilogram of dry matter, for maintenance. The values so derived are contained in the next to the last column of Table 4. In the last column of this table are found the percentages of utilization of the metabolizable energy, obtained by dividing the net energy by the metabolizable energy, per kilogram of dry matter.

The data of Table 4, as already stated, are made the basis for computing the dry matter of feed required for maintenance and the requirement of metabolizable energy for the milking cows. These requirements have been computed as indicated in Table 5.

TABLE 5.—Maintenance requirement of net and of metabolizable energy and of dry matter of feed for the milking cows

Experiment No.	Cow No.	Period No.	Maintenance requirement of net energy	Correction for standing	Maintenance requirement of net energy, corrected for standing	Net energy values for maintenance, per kilogram dry matter of feed	Utilization of metabolizable energy	Dry matter of feed required for maintenance	Metabolizable energy required for maintenance
			<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>	<i>Kgm.</i>	<i>Cals.</i>
221E-----	874	I	6,955	+154	7,109	1,863	77.7	3.816	9,149
	886	I	6,349	+75	6,424	2,003	81.7	3.207	7,863
		II	6,349	+99	6,448	2,003	81.7	3.219	7,802
221F-----	886	II	6,349	+25	6,374	2,003	81.7	3.182	7,802
221G-----	887	I	6,061	+179	6,230	1,871	75.3	3.330	8,274
		II	6,061	+172	6,228	1,871	75.3	3.326	8,264
		III	6,061	+205	6,256	1,871	75.3	3.344	8,308
		IV	6,061	+101	6,152	1,871	75.3	3.288	8,170

The allowances for body gain have been computed on the basis of the observed gains and losses (as indicated in the last column of Table 3) and the corresponding net-energy values of the feed, those for body gain having been determined elsewhere from results of supermaintenance feeding.⁵

TABLE 6.—Feed available for milk production and milk energy per kilogram of dry matter of available feed

Experiment No.	Cow No.	Period No.	Body gain or loss	Net energy per kilogram dry matter for body gain or loss	Dry matter of feed required for body gain or loss	Dry matter of feed required for maintenance	Dry matter of feed available for milk production	Milk energy	
								Total	Per kilogram of available feed
			<i>Cals.</i>	<i>Cals.</i>	<i>Kgm.</i>	<i>Kgm.</i>	<i>Kgm.</i>	<i>Cals.</i>	<i>Cals.</i>
221E-----	874	I	+586	1,520	0.386	3.816	2.677	4,931	1,842
	886	I	+1,544	1,306	1.106	3.207	3.792	6,498	1,714
221F-----	886	II	+1,767	1,306	1.266	3.219	2.505	5,162	2,061
Average (Cow No. 886)		II	+2,384	1,306	1.708	3.182	3.177	5,935	1,888
221G-----	887	I	+371	1,438	.258	3.330	4.445	7,947	1,881
		II	-4,842	1,871	2.588	3.326	3.108	5,589	1,798
		III	-289	1,871	.154	3.344	2.745	5,048	2,058
		IV	+2,581	1,438	1.795	3.288	2.944	6,045	2,053
Average (Cow No. 887)									1,924

In case of a loss of energy by the body, the amount of feed required to prevent this loss was computed by the use of the net-energy value for maintenance given in Table 4. The results in detail are presented in Table 6, which gives also the dry matter of feed available for milk production and the energy of milk produced per kilogram dry matter

⁵ COCHRANE, D. O., FRIES, J. A., and BRAMAN, W. W. THE MAINTENANCE REQUIREMENT OF DRY COWS. *Jour. Agr. Research* (1925) 31: 1055-1082. 1926.

of available feed (last column). The latter data represent the net-energy values per kilogram dry matter of the ration for milk production.

In Table 7 the metabolizable energy available for milk production, and the percentages of utilization of the metabolizable energy in milk production, have been computed in a similar manner.

TABLE 7.—The utilization of metabolizable energy for milk production

Experiment No.	Cow No.	Period No.	Body gain or loss	Utilization of metabolizable energy	Metabolizable energy required for body gain or loss	Metabolizable energy required for maintenance	Metabolizable energy available for production of milk	Milk energy	Utilization of metabolizable energy for milk production
			<i>Cals.</i>	<i>Per cent</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>
221E.....	874	I	+586	61.6	951	9,149	6,834	4,931	72.2
	886	I	+1,544	56.1	2,752	7,863	9,475	6,498	68.6
		II	+1,767	56.1	3,150	7,892	6,936	5,162	74.4
221F.....		II	+2,384	56.1	4,250	7,802	8,689	5,935	68.3
Average (cow No. 886).....									70.4
221G.....	887	I	+371	57.0	651	8,274	11,761	7,947	67.6
		II	-4,842	75.3	-6,430	8,264	7,540	5,589	74.1
		III	-289	75.3	-384	8,308	7,305	5,648	77.3
		IV	+2,581	57.0	4,528	8,170	7,598	6,045	79.6
Average (cow No. 887).....									74.7

The data of Tables 6 and 7 indicate a considerably greater efficiency of utilization of feed energy for milk production than for body production, and seem to show a tendency toward a more efficient utilization of the available feed energy in the later stages of lactation.

The average amounts of milk energy produced per kilogram dry matter of available feed are 1,842 Calories for cow 874, 1,881 Calories for cow 886, and 1,924 Calories for cow 887. The average percentages of utilization of the metabolizable energy for milk production are 72.2 per cent, 70.4 per cent, and 74.7 per cent, for cows 874, 886, and 887, respectively.

The average net energy values of the ration used for maintenance, body increase, and milk production, as obtained with these cows, have been assembled in Table 8 to illustrate the relative utilization of the feed energy for the three purposes mentioned.

TABLE 8.—Relative value of feed energy for maintenance, body increase, and milk production

Cow No.	Net energy value per kilogram of dry matter of feed			Utilization of feed energy		
	For maintenance	For body increase	For milk production	For maintenance (standard)	For body increase (relation to maintenance)	For milk production (relation to maintenance)
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>			
874.....	1,863	1,520	1,842	1	0.816	0.989
886.....	2,003	1,396	1,881	1	.697	.939
887.....	1,871	1,438	1,924	1	.789	.1028
Average.....	1,912	1,451	1,882	1	.761	.985

Regarding the net energy value of the ration for maintenance as the standard of comparison—that is, as unity—and relating to this, respectively, the net-energy values for body increase and for milk production, we find the ratios of utilization of the feed energy as recorded in the last three columns of Table 8.

CONCLUSIONS

In a series of respiration calorimeter studies of the energy metabolism of cows, both in dry condition and in lactation, and on different planes of nutrition, the average rates of utilization of the net energy of the ration for maintenance, lactation, and body increase were found to be as 1 for maintenance, 0.985 for lactation, and 0.761 for body increase. More extensive evidence will doubtless modify this apparent relationship; but accepting the same provisionally, it is certainly of interest, and consistent, from a teleological point of view, that with a lactating female the rates of efficiency of utilization of food for the maintenance of the life of the mother and for the production of milk for the offspring are apparently alike, while the economy of use for body growth is at a distinctly lower rate.



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A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY *APLANOBACTER INSIDIOSUM* L. McC.¹

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INTRODUCTION

In a previous note (4)² the senior writer recorded the finding, in northern Illinois and southern Wisconsin, of a new disease of alfalfa. This disease, chiefly vascular in character, causing as its most conspicuous symptoms a dwarfing and yellowing of the plant, a drying out of the foliage, and occasionally a wilt, has now been under observation and study for a year. The organism found in diseased plants, and with which the disease has been reproduced experimentally, has been studied by the junior writer and been found by her to be a new species of bacteria to which the name *Aplanobacter insidiosum* has been given in a brief description already published (6). The present paper brings together a more complete description of the disease, and such results as have been obtained in an experimental study of the relation of environmental conditions to the development of the disease, and gives a full description of the parasite.

DESCRIPTION OF THE DISEASE

Although the disease has undoubtedly passed unrecognized in the United States for a long time, it is by no means inconspicuous in character, at least in fields where it is abundant and destructive. It is not characterized by any external lesions like those occurring in the bacterial stem blight described by Sackett (11). The most conspicuous character of thoroughly diseased plants is a dwarf habit (pl. 1, A), there sometimes seeming to be an abnormally large number of stems suggesting a witches' broom. Dwarfing is usually more conspicuous when plants have reached one-half to two-thirds growth after the field has been cut. Dwarfed plants are usually paler in color than their healthy neighbors, and their leaves are smaller. On dwarfed stems with short internodes the leaves may be very small and curled upward and yellowed at the margins. In very vigorous fields or in plants recently infected the symptoms may be inconspicuous. Dwarfing may be slight or there may be no dwarfing.

¹ Received for publication Jan. 28, 1926; issued September, 1926. This paper is a joint contribution from two separate offices of the Bureau of Plant Industry of the United States Department of Agriculture. The fore part of the article, beginning on this page and continuing on to page 502, is by Jones, the senior writer, and is devoted especially to the nature, distribution, and control of the disease. The second part, beginning on page 502 and continuing to the end, is by McCulloch, the junior writer, and is especially concerned with the culture and morphology of the bacterium which has been assigned as the cause of the disease.

² Reference is made by number (italic) to "Literature cited," p. 521.

Sometimes a bleaching and drying of the foliage of a few stems or of the margins of a few upper leaves like that following frost injury is the only indication of the disease. In seed fields the disease has sometimes been found betraying itself merely by the yellowing of the margins of the oldest leaves, giving the entire plant an unhealthy appearance. In the greenhouse, infected plants have sometimes shown as first visible symptoms a mosaiclike mottling of the leaves after several hours of sunshine, with a return to uniform color at night. Extreme mottling of the foliage is followed by wilting or shriveling (pl. 2); indeed, the frosted appearance of plants in the fields seems to be the consequence of severe wilting in hot midday. The wilting of older plants has been observed by the senior writer, although it has been described as characteristic chiefly in the first crop, and rarely in the later crop. Wilting of seedlings in their first year has been seen once in the field, and this wilting seems to be the only symptom which young plants exhibit before they perish. The term "wilt," then, as applied to this disease, designates more accurately the relation of the parasite to the host—the extensive clogging of the vascular system described later—than the most conspicuous symptom of the disease in the field.

Whenever this disease has developed sufficiently to be discernible in the foliage, the taproot almost always shows unmistakable discoloration, which is readily observed when it is cut across with a sharp knife (pl. 3). The discoloration, which is yellow or pale-brown in color, is located in the outermost part of the woody cylinder just beneath the bark. The ring of discoloration is narrow at first, increasing in width with the progress of the disease. When the bark is stripped back, the woody vascular cylinder thus exposed is seen as straw-yellow to brownish-yellow in color, very different from the white or ivory-white, rather dry appearance of the same tissue in the healthy plant. This condition is found not only near the crown, but it extends to the ends of long taproots and to the larger root branches. Sometimes in plants which are growing and laying down new wood in spite of the disease, the discolored wood is found deeper than the outer wood beneath the cambium, and is seen readily only in section. In any case, the character and the location of the discoloration distinguish it from the decay of the center of the taproot found so often in older plants.

Another type of root injury which occurs sometimes in badly diseased plants is a reddish-brown, cankerlike lesion in the cortex and extending more or less deeply into the wood and seen best when the bark is stripped back from the wood (pl. 1, B). This injury is rarely observable from the outside of the root. The brown discoloration of the wood extends up and down from these lesions, which appear to mark a point of entry of the parasite. This type of injury occurs from the crown to a depth of 6 to 8 inches in the soil, and is less readily distinguished from other types of crown decay.

Dwarfing and yellowing of plants, or bleaching of the foliage, together with discoloration of the outermost woody tissue of the taproot, are the distinguishing characters by which this disease can usually be easily recognized.

The distribution of the disease in the field may be uniform'y scattered, especially if the field is level and uniform, but more frequently it occurs in patches. It is always more abundant along depressions

where surface drainage takes place, and along irrigation ditches. Frequently from isolated beginnings it spreads conspicuously in the direction of surface drainage or in the direction of the flow of irrigation water.

DISTRIBUTION OF THE DISEASE

The disease is now known, locally at least, in many States where alfalfa is grown. It has been reported recently in correspondence from New Jersey, Pennsylvania, and South Carolina, by J. L. Weimer. In a preliminary survey in May and June, 1925, by Weimer and the senior writer (5) the disease was observed in Alabama, Mississippi, Indiana, Illinois, Wisconsin, South Dakota, Nebraska, Kansas, and Colorado. The pathogenicity of cultures of the parasite obtained from plants in all of these States, except Indiana, has been determined by inoculation of plants in the field and the production of typical disease. Recently the disease was found by M. B. Linford in Cache Valley, Utah, and by the senior writer and B. L. Richards in southern Idaho. This experience suggests that further search probably will result in discovery of the disease in greater or less abundance in many more if not all of the older alfalfa-growing districts of the United States.

PREVIOUS RECORDS AND STUDIES

References to this disease that can be identified with certainty are few and are found only in recent writing. Headden (3) gives an account of alfalfa failure in Colorado, which does not give a convincing description of this disease; yet representative dying plants sent to the senior writer were excellent specimens from which cultures of the parasite were obtained. Sackett (12) has written a description of the disease that seems unmistakable, and Durrell and Sackett (1) later describe it even more clearly. To be sure, the bacterial organism which these latter writers found associated with the disease appears, from the one cultural characteristic given, to be different from that described in this paper; but complete proof of the ability of their organism to reproduce the disease as found in the field was not given by them in their note (1), and until such proof has been obtained it is not possible to distinguish the disease found by Durrell and Sackett from that described in the present paper. Melhus et al. (8) record an experimental study in Iowa of water-flow interference in roots of alfalfa suffering from a wilt disease, which, from the brief description they give, appears identical with the one discussed in this paper.

ECONOMIC IMPORTANCE OF THE DISEASE

It is not possible to state with certainty to what extent this disease is responsible for decrease in alfalfa culture and for "failures" which seem to be reported in increasing numbers in several parts of the United States. In the past the dying of plants from this disease has been frequently ascribed to winter injury; and more recently, especially in Colorado (1), when parasitic nematodes have occurred associated with the disease, the injury has been ascribed entirely to the nematodes. Much further field study will be required before a reliable

appraisal can be made of the relative importance of winter injury and this bacterial disease wherever the two troubles occur together. In the course of a field survey made during the summer of 1925, fields of alfalfa 3 to 5 years old were found in Alabama, Wisconsin, Illinois, Kansas, Nebraska, and Colorado that were greatly reduced in stand and made unprofitable by this disease. In Kansas and Nebraska this observation has been supported by Melchers (7) and Goss (2), respectively. There is, therefore, no doubt that the disease, under certain conditions, is capable of destroying fields in localities in all of these States, yet nowhere has it been found occurring in all fields in a locality—on the contrary, sometimes in only a small percentage of them—and neither is it destructive in all of those in which it occurs. A discussion of conditions which seem to favor destructive spread of the disease is given later.

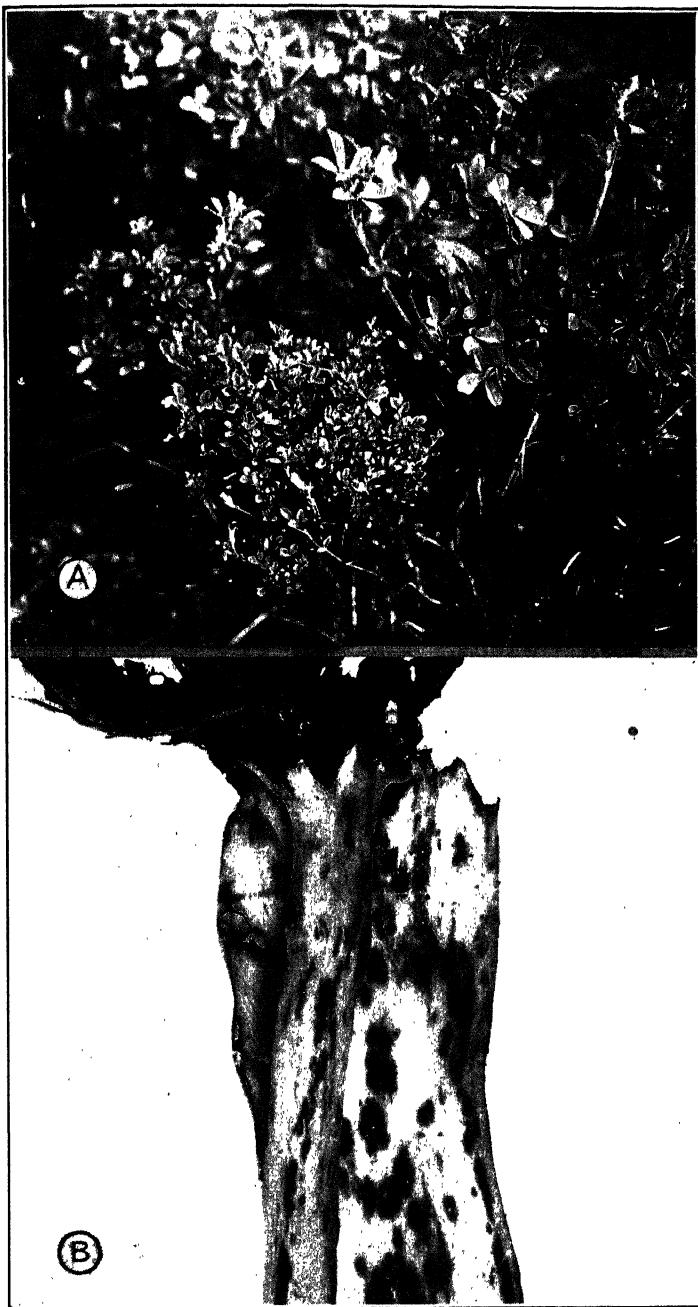
RELATION OF THE PARASITE TO THE HOST

Study of the relation of the parasite to the host—the routes by which the parasite gains entrance, the manner of its spread through the tissues, the conditions under which the disease progresses to cause the death of the plant, and the bearing which age may have on the response of plants to infection—has not reached satisfactory conclusion. Such study involves a more exact knowledge of the anatomy of the plant and the effect of environmental conditions upon its growth than is available at present. Therefore the present account of these relations is merely a statement of the more obvious features that have been observed and examined.

METHOD OF INFECTION

Direct inoculation of alfalfa plants with pure culture of the parasite has been made successfully by two methods, both involving wounding of the host. The first method introduces the organism directly into the vascular system of the plant; the second brings the organism into contact with wounded parenchyma through which it must pass to enter the vascular system. The organism has not yet been found to enter uninjured tissue.

The first method, used almost exclusively in experimental inoculations thus far, takes advantage of the great avidity of the freshly cut vascular tissue of alfalfa root or stem for water. Alfalfa stems have never been observed to "bleed" when cut, but even when the soil is wet they take up water, and this water may carry bacteria in suspension a considerable distance into the plant. The conditions most favorable for absorption of water and the extreme distance to which bacteria may be carried have not been determined. Bacteria may be carried both upward and downward from cuts. In a preliminary demonstration, a vigorous shoot of alfalfa 38 centimeters tall was cut off close to the crown, the cut being made under a bacterial suspension in which the stem was left standing in the greenhouse for 5 hours before it was examined for the presence of bacteria. Razor sections from 3 centimeters above the cut showed vessels to be clogged with pale-yellow masses of bacteria, and bacteria were obtained in plates poured from the eighth centimeter of stem above the cut. In a younger stem 22 centimeters tall, bacteria were found 6 centimeters above the cut 5 hours after the stem was cut off. Similar

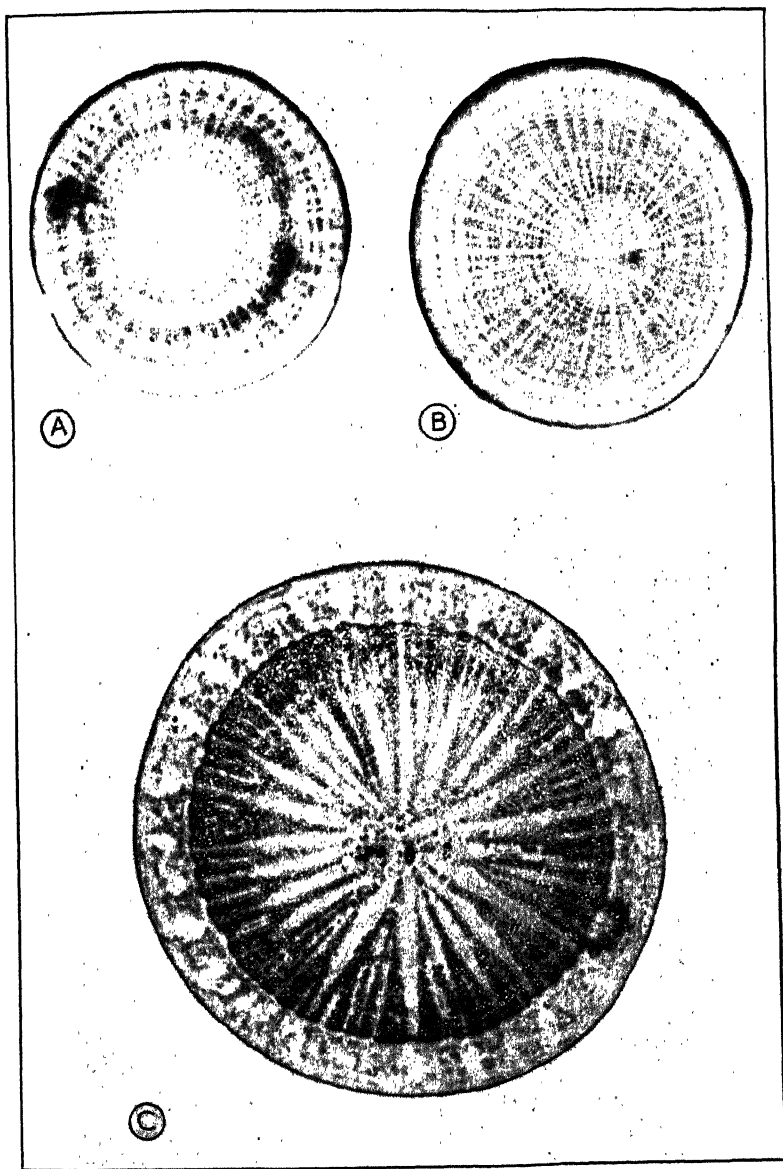


A. Alfalfa plant severely diseased with bacterial wilt and root rot (lower left), showing characteristic appearance in the field about two weeks after cutting. The foliage is very small and pale compared with that of the healthy plant at the right.

B. Two-year-old alfalfa root infected with bacterial wilt. The bark has been cut and separated from the woody cylinder to expose abundant reddish-brown lesions, some of which appear to mark the point of entry of the parasite into the root through lesions about injured and killed rootlets.



Disease symptoms obtained in the greenhouse by inoculation with a culture of *Aplanobacter insidiosum*. Two uninoculated plants are shown in the group at the left. Inoculation was made October 8, 1924. Photographed January 19, 1925. The plants were not cut back in the meantime, and therefore show none of the dwarfing that is so conspicuous at a later stage in the development of the disease

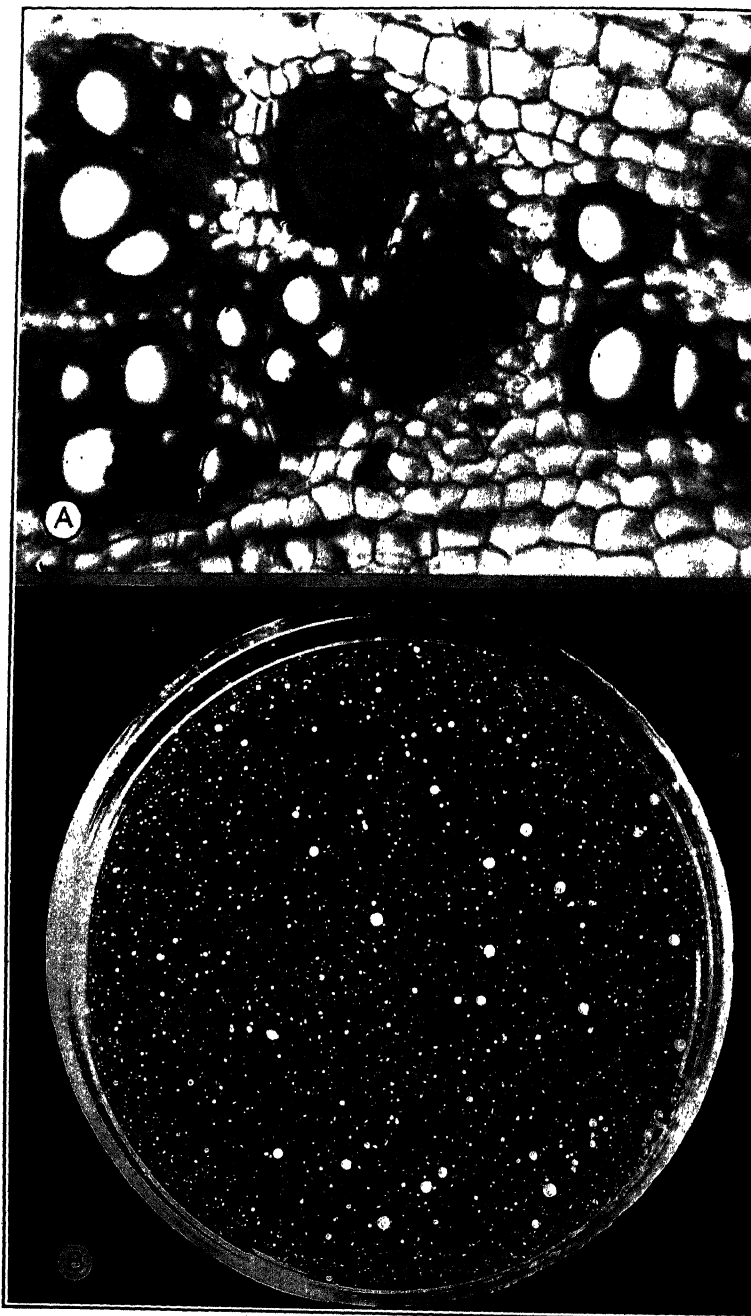


Discoloration of root in the outer wood beneath the bark characteristic of bacterial wilt and root rot

A. Cross section of an infected plant 3 years old. Note in the outer part of the wood the discoloration which in this case (as often) is not of equal intensity around the entire circumference of the root. $\times 3\frac{1}{4}$

B. Cross section of healthy root 3 years old. $\times 3\frac{1}{4}$

C. A thinner section of the diseased root shown in B photographed by transmitted light to show more clearly that the discoloration which appears diffused in B is due chiefly to the colored material in invaded vessels. $\times 6$



A. Three vessels of the vascular system of alfalfa plugged with bacteria are shown in the center of the photograph. $\times 250$
 B. *Aphanobacter insidiosum*. Plate for isolation. Nine-day-old culture on beef agar. Note the small size of the colonies at this age. Actual size

stems cut near the top under bacterial suspension and allowed to remain in the suspension four hours, gave abundant bacterial colonies from the ninth centimeter below the cut, which was the greatest distance from the cut at which examination was made.

Bacteria are carried a considerable distance in a much shorter time than that used in the foregoing trials. Large stems of plants in late bloom growing in the field were cut off about 15 centimeters above the crown, with a razor wet with bacterial suspension, and the cut surface was quickly irrigated with a single drop of the suspension as large as the level surface would hold. This drop was immediately absorbed. Five minutes later the stump was cut off, then sterilized on the surface, and cut into pieces one-half centimeter long which were macerated and plated. An abundance of bacteria was obtained from the fourth centimeter below the top of the stump. This experience indicates that the host plant would readily take the parasite into its vascular system when wounded in mowing or cultivation. Nearly all of the inoculations made thus far have been made by cutting stems or roots under bacterial suspension or with a razor wet with bacterial suspension, and in no case has infection failed to result.

The second method of inoculation, tried with pure culture but once in the field, consisted in removing the soil from around the crown and scraping the bark of the taproot, taking care not to penetrate the wood, and then applying a bacterial suspension to the scraped surface. This inoculation was made July 17. On September 22 ten of the inoculated plants and 10 of the injured but uninoculated controls were removed for examination. The uninoculated plants had healed, and showed no deep injury. The inoculated plants, however, while showing little injury on the surface, had reddish-brown lesions in the cortex beneath the wounds extending down into the wood. Vascular infection occurred in all of these plants, sometimes only immediately above and below the discolored spot in the wood. It was thought that some of the small roots might possibly have been cut accidentally by the instrument when the taproot was scraped and that the infection might have gained entry into the taproot in that way, so slides were made of the discolored cortex to find out whether the vascular tissue of any of the small roots had been cut, but examination of the slides failed to reveal any such avenue of access for the infection into the taproot. From eight lesions isolations were made from cortical tissue only, and in seven cases abundant colonies of the parasite were obtained. From this it appears that the parasite can make its way through parenchyma from superficial wounds to the vascular tissue, causing more or less cortical crown or root rot. These reddish-brown lesions have been found in abundance in one 3-year-old field of alfalfa which was dying very rapidly from this disease, and the parasite has been isolated readily from such cortical injury. This type of injury by the parasite deserves further study.

DISTRIBUTION OF THE PARASITE WITHIN THE HOST

Whatever the manner in which the parasite gains entrance into the vascular system, it travels in that system slowly, passing through the crown from root to stem or from stem to root with equal facility.

The bacteria in the vessels may be seen in razor sections as a more or less deeply colored yellow mass filling the entire cavity (pl. 4, A). The cell wall and also the contiguous wood fibers usually remain free from color, at least for some time. The yellow material in the vessels becomes denser and darker in color with age, until the lumen of the cell is filled with a yellow gum. Whether this final material is of bacterial origin or is deposited by the plant is not known.

The vessels of the root that are filled with bacteria are usually the outermost ones, those nearest the cambium, and are more or less densely scattered around the entire circumference. In older roots they are rarely found in vessels near the center. From superficial examination it appears that the reason for this distribution is that the bacteria, whether they enter through the cortex or come down from stems, find their way into, or have the most unobstructed entrance into these outermost vessels, which anastomose freely around the entire circumference but which connect radially only rarely if at all. In the stems there appear to be no tangential connections; the bacteria in passing upward apparently follow only those vessels which they entered at the base. In fact, all of the sections of stems that have been observed have shown a diminution in the number of clogged vessels from internode to internode progressively upward.

Invasion of the stem seems to follow the maturity of the tissue. Little or no invasion has been found in young shoots, even when they show conspicuous evidence of disease, which suggests that disease symptoms are largely if not wholly the result of bacterial action in the roots. Extensive invasion of stems has been found, both in the greenhouse and the field when there was very little or no evidence of disease in the foliage. Stems grown in the greenhouse from roots inoculated by dipping ends of taproots which were cut 10 inches below the crown in bacterial suspension and grown under favorable conditions have yielded cultures of the parasite from petioles of leaves 3 feet above the point of inoculation before visible symptoms have appeared.

DOES VASCULAR INVASION EXTEND TO THE SEED

Some of the plants described above blossomed abundantly, but even with hand pollination they set only a few seed pods. Six of these plants were examined as soon as mature, by plating the pedicel, the pod, and the seed removed aseptically from the pod. One pedicel gave a few colonies of the parasite, but none were obtained from the pods or seed. The few remaining pods were accidentally lost.

The fact that bacteria have been found in leaves and so close to seed suggests strongly that they may at times enter the seed itself, and in this way be carried to new fields in viable seed. In order to explore this possibility further some days were spent in late August and early September in seed fields in Kansas and Idaho where the disease was found in greater or less abundance in seed plants. As was expected, some of the severely diseased and stunted plants in the field produced no blossoms, while numerous others produced blossoms but set no seed. A few infected plants were found that showed but slight evidence of disease in the foliage, and were vigorous enough to mature more or less seed.

The collection of infected plants bearing seed and taken to the laboratory for study has not yet been completely examined; but thus far the parasite has not been isolated from the seed.

EFFECT OF TEMPERATURE ON THE PROGRESS OF THE PARASITE THROUGH THE PLANT AND ON THE DEVELOPMENT OF DISEASE

In a preliminary inoculation in the greenhouse, 2-year old alfalfa plants transplanted from the field early in January and now in blossom were inoculated by cutting back the stems close to the crown with a razor dipped in a bacterial suspension. Five pots containing three inoculated plants each, and two pots with uninoculated plants, were placed in each of three greenhouse compartments maintained, with only a little fluctuation, at 16°, 24°, and 28° C. Inoculation was made March 2. On March 23, no evidence of disease having appeared in the foliage, the tops were cut back. By April 2 inoculated plants in 2 pots held at 28° showed typical symptoms in dwarfed foliage, and 4 inoculated plants held at 24° showed less advanced symptoms. On May 4 all remaining plants were removed from the soil and examined. Twelve of the 15 inoculated plants that were held at the highest temperature (28°) showed more or less vascular invasion in the taproot; a like number showed no infection at the intermediate temperature (24°); while all plants held at the lowest temperature (16°) were infected, but they showed relatively little vascular invasion and no evidence of disease in the foliage at any time.

A more critical study of the effect of temperature upon the progress of the parasite through the vascular system was attempted in the Wisconsin soil-temperature tanks. Alfalfa roots similar to those used in the preceding experiment were cut off about 10 inches below the crown and inoculated by standing them in a bacterial suspension when they were transplanted from the field. Inoculation made in the root permits a more satisfactory control of temperature at the point of inoculation than inoculation at the crown. Transplanted roots placed immediately at high temperature rot easily; most of one series of plants placed at temperatures from 9° to 33° C. was lost on this account. The upper limit of temperature at which the parasite will flourish in the host has not been determined.

Another group of plants inoculated as in the preceding trial were transplanted in like manner and placed in temperature tanks held at 9°, 12°, 15°, and 18° C., to determine the lowest limit of temperature at which advance through the vessels takes place. Three plants were placed in each can, and four cans were placed at each temperature. Inoculation and planting was done January 14. At the end of January, one of the plants held at 18° had bacteria in abundance in the lower half of the root, but few in the upper half. On February 11 a number of vessels in one of the plants held at 18° were found plugged close below the crown. In one of the plants held at 12° only a few bacteria were present even 4 inches above the cut end. On March 3 bacteria were isolated from the petiole of a leaf more than 2 feet above the ground on a plant grown at 18° soil temperature. At mid-April it was no longer practicable to maintain a soil temperature at 9°. At this time one plant in this series showed slight dwarfing typical of the disease, but in none of the stems were bacteria found more than 8 inches above the crown. Thus even at this low temperature of 9° bacteria had advanced through the root into the stems, and had been able to produce some slight symptoms of disease. So there was no temperature in this series that was low enough to prevent the development of the parasite in the plant.

When all of the plants were cut back on May 10 none showed striking symptoms of disease. Shortly after this the plants were accidentally allowed to dry up. Examination showed, however, that new growth of wood outside the diseased tissue was taking place, indicating a partial or temporary recovery.

Thus it appears that bacteria pass through the vascular tissue of the root at least slowly, even at a soil temperature as low as 9° and readily at 12° or 15° C.; but under the conditions of this trial none of the plants were killed in five months—even the symptoms of disease were not striking—and new uninvaded wood growth produced on the outside of the taproot suggested that these plants were likely to live and perhaps to thrive for a long time.

Further study of the effect of temperature upon the passage of bacteria through the host tissue must be made; but it is even more important, though certainly more time consuming, to determine the effect of temperature upon the longevity of infected plants.

HOST PLANTS

The only legume growing in close association with diseased alfalfa which has shown disease with similar symptoms is sweet clover. Sackett (12) states that alfalfa and sweet clover are both affected in Colorado. This statement seems to be based on observation of symptoms rather than upon any experimental demonstration of the common origin of the disease in the two plants. In preliminary trials which have been made to inoculate white sweet-clover plants with the alfalfa parasite disease has not been produced.³ A few plants of red clover and of several annual legumes have been inoculated by introducing the alfalfa parasite into the vascular system; but in no case has it made extensive growth in these plants or produced symptoms of disease.

DISCUSSION

Although a discussion of the importance of this disease and its relation to climatic factors based on observations made during a single season as described previously is necessarily a record which may need modification later, nevertheless it may be worth while to record those impressions. Bacterial wilt of alfalfa has not been found thus far a disease of seedlings or young plants, but chiefly of plants 3 years old or older. For the most part it occurs more frequently in alfalfa fields in regions where this crop has been grown for a considerable number of years. The disease also appears to increase in abundance and also in the rapidity with which it spreads through fields and destroys plants in a direct relation to the abundance of water supplied to the surface of the soil. Thus, although the disease may occur in occasional plants in old fields where annual rainfall is as low as 20 inches, it appears rarely to be destructive until rainfall exceeds 25 inches. Nearly all of the badly diseased non-irrigated fields have been in territory with rainfall between 30 and 40 inches. When water is supplied by irrigation, the disease has been found thus far absent or very scarce where fields are subirrigated, the surface soil remaining dry during most of the summer.

³Since the above was written, infection of white sweet clover has been secured readily in the greenhouse, and a wilt of sweet clover produced by *Aplanobacter insidiosum* has been found in one Wisconsin field where the sweet clover seed was sown among diseased alfalfa plants.

When surface irrigation is used, the disease has been found largely along irrigation ditches in the more porous soils. It is more abundant throughout fields when soil is retentive of moisture, remaining wet a relatively long time after water is applied.

If these generalizations are confirmed in following years, then it appears that this disease will never become important in the drier part of the United States where alfalfa has been most extensively grown in past years; but increasing trouble may be anticipated in the more humid regions, especially in the central Mississippi Valley, where the crop has been grown more extensively in recent years.

CONTROL MEASURES

Until the life history of the parasite has been completely traced, and until the conditions which favor infection and the rapid death of the plants are better known, control measures can be suggested only for trial. The absence or scarcity of the disease in the drier part of the alfalfa-growing area of the United States indicates that the disease is not likely to become established or injurious there in the future. Effective control is of interest in those irrigated districts where the disease has already appeared, and in the warmer and more humid States where the disease threatens to check the present expansion of alfalfa culture.

Although the transmission of the parasite within or with the seed has not yet been demonstrated, it must still be reckoned as a possibility; and therefore seed from disease-free fields should be chosen if possible. The present knowledge of the distribution of the disease is so incomplete that it can not be said with certainty that the disease is absent from any seed-growing district, although in most districts it probably occurs in only a small number of fields. Generally speaking, the climatic conditions most favorable for seed production seem unfavorable for the development of this disease, so in any case the amount of diseased seed on the market can not be large. Whether the parasite is carried with seed or not, it is undoubtedly carried in a living condition for a long time in alfalfa hay. The survival of the organism for five months in hay has been demonstrated, and beyond doubt it lives much longer. Such hay scattered in new fields could undoubtedly, under favorable conditions, be a source of infection.

After the disease has appeared in a field it seems to be distributed principally by natural agencies—e. g., the flow of surface water—agencies which can hardly be controlled. However, in one instance, the most rapid distribution seemed to be in the direction taken by the mower across a diseased strip. Presumably the mower in cutting diseased stems became smeared with the bacteria, which were carried to the cut stems of healthy plants. Spreading by the mower will undoubtedly be greatly lessened if fields are cut only when they are dry.

In irrigated fields the disease has been observed to spread with the flow of irrigation water over a group of diseased plants. When the disease has appeared in an irrigated field care should be taken to use no more water than is necessary, and any cultivation of the field which wounds the crowns when the soil is wet should be avoided.

The length of time during which the parasite will persist in the soil after a diseased alfalfa field has been plowed has not been determined.

However, disease in new plantings on old fields has been observed in several instances to have spread from old diseased crowns that survived an intervening period of cultivation. Fields where the disease has been should not be replanted to alfalfa until all the old plants which can carry such infection are dead and thoroughly disintegrated.

CULTURAL AND MORPHOLOGICAL CHARACTERS OF APLANO-BACTER INSIDIOSUM L. McC.⁴

The junior writer's study of this organism began several months after the senior writer discovered the alfalfa disease in Wisconsin, and after he had made isolations and accomplished infections with it that proved it to be of bacterial origin (4). Bacteria isolated by the senior writer and diseased alfalfa plants were sent to Washington, D. C., where the following study of the characteristics of the organism was made in the Laboratory of Plant Pathology of the Bureau of Plant Industry of the United States Department of Agriculture.

A brief description of the organism and the name selected has already been published (6).⁴

The senior writer provided several lots of diseased alfalfa plants from Wisconsin. Some of these were field plants with natural infection, and others had been inoculated by him. Isolations made in the Washington laboratory from these plants consistently produced the same type of bacteria that the senior writer had isolated in Wisconsin.

In order to have definite proof of the pathogenicity of the organisms isolated by the junior writer, a number of healthy alfalfa plants growing in the greenhouse in Washington were inoculated. Typical infections were obtained in every case, and the reisolated organisms were identical in character with those used for the inoculations.

The thought that this disease might possibly be caused by *Bacterium solanacearum* or *Bacterium medicaginis* was soon dismissed, as the organism isolated from the diseased tissues showed characters distinctly unlike either of those organisms or any other described plant pathogen.

BACTERIA IN THE PLANT

In infected plants that show but slight outer manifestations of disease the vascular system often contains bacteria from the tip of the root to the very top of tall stems. Recently infected tissues show no discoloration or other unusual symptoms to the naked eye, but a microscopic examination reveals bacteria in the vessels. In older infections many vessels are filled with a yellow to brown, fine granular or gumlike substance (old bacteria?), and others are filled with the colorless bacteria. The bacteria do not diffuse very readily in the water of the mount; but in heavy infections enough bacteria escape to form a milky clouding about the sections. The bacteria are small and nonmotile; and these characters, together with the location of the bacteria in the vessels, make diagnosis of the disease comparatively easy.

⁴In Migula's (9) classification the genus name becomes *Bacterium*. In the classification recommended by the Committee on Bacteriological Technic of the Society of American Bacteriologists (13) the genus name is given as *Phytomonas*.

ISOLATION OF THE BACTERIA

In selecting tissues for isolation purposes, it is best to choose, if possible, those without dark-colored vessels. The older, dark tissues are more likely to contain secondary organisms.

Two methods have been used in making isolations. In one the woody cylinder is removed with sterile instruments without previous surface sterilization.

The other method, which is much easier and just as successful, is to take the selected root or stem (at least an inch in length), remove the surface irregularities, put it into 95 per cent alcohol for 3 to 5 seconds, then burn off the alcohol and rinse in water, then with sterile instruments remove and discard the cortex. Then, whichever method has been used, the central woody part should be cut or shredded into small bits, put into broth, and shaken well several times before plating. The agar for the isolation plates may be inoculated directly from the bouillon containing the finely divided tissues, or from dilutions. Rather heavy inoculations should be made, for although the bacteria may be present in great numbers, many of them are apparently dead, or much reduced in vitality. Usually 10 centimeters or more of uninoculated agar is poured into the plate, and then the tube of inoculated agar poured on top of this. The additional media keeps the culture from drying out while the slow-growing colonies are developing.

Plates should be kept under observation for at least 15 days. The colonies are seldom visible before the fifth day, and in a number of instances they were not visible until several days later (pl. 4, B). In one case the colonies were not visible until the thirteenth day. A temperature of 20° to 23° C. is the best temperature for growth.

Pure cultures of the alfalfa bacteria have been obtained in many isolations, but frequently there are rapidly-growing antagonistic white bacterial colonies along with them which absolutely inhibit the growth of the alfalfa bacteria within a radius of 10 to 15 millimeters. With several of these antagonistic colonies scattered over a plate, it is useless to expect any of the alfalfa colonies to appear. This intruder has been found more often in plates from roots than from stems.

The alfalfa organism has been isolated repeatedly by the junior writer from plants grown and artificially infected in Washington, and from plants sent to Washington from Wisconsin. Later in the season the senior writer sent diseased plants from Alabama, Colorado, Illinois, Kansas, and Mississippi, and the organism was isolated from all of them.

Successful isolations have been made from alfalfa stems that had been pressed and kept at room temperature for five months. There was apparently no reduction in numbers or vitality.

Colonies selected from the different isolations have been compared and studied in regard to morphology and cultural characters.

MORPHOLOGY

In the host plant and in cultural media, the organism is a short, nonmotile rod, rounded at the ends, occurring singly and in pairs. No chains have been found in any medium. Single rods are 0.7 to 1 by 0.4 to 0.5 μ ; the paired rods are 1.8 to 2 by 0.4 to 0.5 μ . The

size varies considerably in any given culture, but difference of media does not seem to affect the size; nor does the age of the culture, within reasonable limits, affect the size, except that in quite old cultures there is a larger proportion of small sizes. Irregular shapes are found occasionally in most media, and in Dunham's solution about half of the bacteria are lunate or some other unusual shape.

The organism stains well with Ziehl's carbol-fuchsin, gentian-violet, Loeffler's methylene blue, methyl-violet, dahlia, and safranin. No spores have been found. Capsules are formed in most media. They are best demonstrated from young beef-agar cultures stained with Ribbert's dahlia stain.

Much time has been given to examinations for motility. Cultures of various ages grown on various media and at various temperatures have been examined. Occasional paired rods are seen which appear to have a slight motility; these swing about in a half circle, turn over, and sometimes change position slightly with reference to other rods. These motile (?) individuals are rare, and so far all attempts to demonstrate flagella have failed. With the Casares-Gil method, one colony (2001) has twice shown a few rods with a single, very short projection or line of small dots at one pole. These do not look like flagella but rather like a bit of slime. The growth is viscid, and it may be that these structures are projections or parts of the capsule. With Van Ermengem's method no better results were obtained. The organism is Gram-positive, and is not acid-fast.

CULTURAL CHARACTERS

The following cultural characters have been described from cultures grown at or as near 23° C. as possible, as this seems to be the most favorable temperature for the organism. The beef media were made from beef infusion, unless otherwise stated. The P_H values were adjusted to 6.8 or 7. Ridgway's color standards (10) have been used.

The organism is slow in growth; and, except on sugar media, the amount of growth is only scanty to moderate, and quite often a set of cultures fails to grow at all. This uncertain character of growth necessitated the repetition of many experiments. Solid media are more favorable for growth than liquid. In many cultures the bacteria seem reluctant to grow in the moister parts of the tube.

BEEF-AGAR⁵ POURED PLATES

On beef agar the growth is very slow, and the colonies do not attain a visible size for several days. The time of appearance depends on the vigor of the organism, the temperature, and the medium. When plated directly from diseased alfalfa tissues, in beef infusion agar of a favorable P_H value (6.8 to 7), and kept at a favorable temperature (22° to 24° C.), the colonies are usually visible with a hand lens in five days. When plates are inoculated from cultures that have become accustomed to artificial media, the colonies appear in three to four days. Under unfavorable conditions the growth is so slow that the agar often dries out before the colonies

⁵ All beef media used were made with beef infusion plus 1 per cent of Difco peptone.

reach a visible size. If the medium retains sufficient moisture, colonies may appear in the plates 10 or more days after inoculation.

The surface colonies are at first white and rather thin. If well separated and with other conditions favorable, they become thicker and yellow. The surface color is pale-yellow, the reverse side being deeper in color (Naples-yellow). The colonies are circular, smooth, and shining; flat to slightly convex; margins entire. The structure is homogeneous; under the lens or microscope, finely granular. Occasionally, in unusually vigorous growth, the interior has numerous, small, irregular, transparent cracks, rather generally distributed through the colony. After a few days these fill in with the usual, fine, granular, nearly opaque growth. Submerged colonies are dense, opaque, yellow; mostly elliptical or spindle shaped; coarsely granular under the microscope. Well isolated surface colonies attain a diameter of 7 millimeters; one measuring 9 millimeters was found. In average platings the colonies are 2 to 4 millimeters in diameter when 9 to 10 days old (pl. 4, B). The growth is viscid, yet soft enough in some cases to flow over the margin of the colony when the plate is placed on edge. When old and dry, the colonies, both surface and buried, are transparent and pale-amber. Very rarely an old colony has in the center a few specks of blue color, visible only with a lens.

BEEF AGAR PLUS 1 PER CENT DEXTROSE, POURED PLATES

Growth is encouraged on this medium. Some surface colonies are 0.5 millimeter in diameter on the fifth day, and 5 to 6 millimeters on the ninth day. The characters of growth are the same as in plain beef agar, except that the growth is more rapid and less viscid or not at all viscid.

When two to three weeks old, if the medium has not dried out too much, some of the well-isolated surface colonies become considerably changed in color by the formation of blue and violet pigments in the growth. The brightly colored granules most often develop in the center of the colony, or in a wide border about the center. Usually there is a blue central area surrounded by a narrow violet zone. Sometimes the colony has either blue or violet alone, and sometimes both colors occur irregularly scattered through the colony. In colonies 4 to 6 millimeters in diameter the colored spot varies in size from a mere point to an area 3 millimeters in diameter, and the colors persist, even in old, entirely dry plates. The colors show best on the lower side of the colony. Seen from above through the yellow growth the colors are less vivid, often merely bluish or greenish gray. Small or crowded colonies rarely develop these colors, and even in the larger well-isolated colonies the formation of color is uncertain. In adjacent colonies that apparently have the same conditions, one may have only blue, another only violet, another both colors, and a fourth no color.

POTATO AGAR PLUS 1 PER CENT DEXTROSE, POURED PLATE

This is a favorable medium, and growth on it is as good or better than that in beef agar plus dextrose. Colonies often are 6 millimeters in diameter five days after becoming visible. They are almost color-

less at first, like boiled starch; the surface is smooth, and the interior is full of irregular, transparent cracks which later disappear. Blue and violet colors develop as in the colonies on beef plus dextrose.

YEAST-AGAR POURED PLATES

Colonies in this medium are more elevated than those on most of the other media, small crowded colonies being practically hemispherical in elevation; opaque; Naples yellow. Submerged colonies are spherical, lenticular, or angular in shape; those near the surface are usually large and often bursting out with growth on one side. This medium retains its moisture rather better than beef media, the growth continues longer, and the blue and violet colors are less likely to appear in the plate growth than in tube cultures on the same medium.

WHEY-AGAR POURED PLATES⁶

Colonies on this medium are more elevated than those on beef or potato agars but less than those on yeast agar. The colonies enlarge rapidly and coalesce to form large areas of smooth, shining growth, which is at first nearly colorless and contains numerous transparent cracks but later becomes yellow and practically opaque. Blue and violet granules develop still later, as in beef plus dextrose plate colonies.

BEEF-AGAR SLANTS

Inoculations made from liquid cultures produce a uniform layer of growth, very thin and colorless for several days, becoming moderately thick and pale yellow. In the V there is a somewhat thicker area, and it is deeper in color (Naples-yellow). Streak inoculations made from agar cultures produce a moderately thick growth along the inoculated line, with a slow outward spread of thin transparent growth having entire, smooth margins. Growth is never abundant, and when some weeks old (at room temperature) it is thin and transparent, except in the small, thicker area in the V. Cultures at lower temperatures (4° to 7° C.) and subject to less evaporation, retain a moist, almost opaque growth for at least five months. The growth is viscid. With a loop it can be stretched out 30 to 40 millimeters before one end or the other loosens and contracts like a bit of rubber. At room temperature this viscosity gradually diminishes after about the twelfth day. There is no clouding below the V. There is no odor, and the medium does not change in color. When old, a very few cultures have had a trace of blue in the V region, and this was found only with the aid of a lens. The growth is fine granular in structure. Pseudozoogloae have been observed in old cultures, but they are smaller and less noticeable than those in agar containing sugar.

BEEF-AGAR STABS

There is a moderate amount of pale-yellow, smooth surface growth which sometimes is soft enough to flow like a thick liquid. The stab remains visible as a fine granular line of no particular character.

⁶ Fresh milk. Boil gently for 5 minutes, then add drop by drop 20 per cent HCl until all the casein is precipitated. Remove the coagulum by straining through cheesecloth. Neutralize the whey and add to each liter: 300 c. c. H₂O, 3 gm. gelatin, 15 gm. cane sugar, 15 gm. peptone, 15 gm. agar. Steam $\frac{1}{2}$ hour, clear with egg albumen, filter, tube, and autoclave.

Neither slant nor stab cultures in beef-extract agar gave growth as good as that on the beef infusion media, and the growth was not viscid.

BEEF AGAR PLUS 1 PER CENT DEXTROSE

On this medium growth is more abundant than on plain beef agar. The thick, but not entirely opaque, smooth Naples-yellow growth covers the surface of slants or stabs, and the V of slants fills with growth. In young vigorous cultures there are small, irregular, transparent cracks in the growth. Growth extends downward between the tube wall and the medium. In stabs the needle path remains visible as a dense granular line. There is an acid reaction from the dextrose, which causes a slight greening of the medium. This acid condition disappears later, and a blue color begins to replace the yellow color of the growth until the bacterial growth in the lower part of slants is dark blue-gray.

BEEF AGAR PLUS LACOTSE

Growth is much like that on beef and dextrose, except that the blue discoloration begins earlier and becomes more abundant.

YEAST AGAR

This medium is very favorable for growth. The surface becomes covered with a moderately thick, smooth, shining, Naples-yellow growth. Blue and violet flecks have been observed as early as the fourth day, but usually this discoloration does not begin until later; in some cultures no blue was seen until after five weeks. Eventually the blue granules that cause this change in color are so numerous that the growth becomes dark blue, with the original yellow color visible only at a few edges or in the lower part of the V. On old, rather dry cultures, the dark bacterial surfaces have a copper to red iridescence. In stab cultures the stab remains visible as a granular line. Numerous, spindle-shaped pseudozoogloecae develop in old cultures (pl. 5, B).

WHEY AGAR

Growth is more abundant on this medium than on any of the other media used. On the slants it is thick, opaque, smooth, Naples-yellow. In the V of slants, where there is a thicker accumulation, it is mustard-yellow. In young cultures the growth is full of small, irregular, transparent cracks. Heavy clouding extends along the wall below the V to the base of the tube. The cultures remain moist much longer than in other agars. Cultures kept at room temperature for three months still retained some moist areas of growth. The blue discoloration is also very striking. Large areas, and often the whole of the growth, become dark blue. The medium is slightly greened. Numerous spindle-shaped pseudozoogloecae are found in old cultures.

POTATO AGAR

Growth is moderate, and the blue granules develop as readily and as abundantly as they do in cultures on potato agar to which sugar has been added, but the general color is less blue. Payne's gray and slate-gray are close matches for the color of the cultures. In

some slant cultures a fugitive blue-green color was observed in the medium before the growth became visible. This color is probably of the same nature as that often seen on potato cylinders before the bacterial growth becomes visible.

POTATO AGAR PLUS DEXTROSE

On this medium the growth is only moderate, being thin and colorless at first, then becoming thicker and pale yellow. The medium is greened slightly. In stab cultures the medium just below the surface (upper 4 to 5 mm.) becomes opaque, as though some growth had invaded the medium. This opaque area is irregular at the lower margin. In slant cultures there is a moderate amount of clouding below the V along the tube wall. No pseudozoogloae have been observed. As in other media containing dextrose, blue granules develop in the growth. The blue discoloration is considerably less than in whey and yeast agar cultures, and it often consists only of rather thinly scattered specks. Other cultures may have fairly large areas of gray-blue color, and under the lens bright blue and green colors are seen.

POTATO AGAR PLUS LACTOSE

There is better growth and more blue color on this medium than either the plain potato or potato plus dextrose. The whole of the growth layer becomes dark blue, with just a line of yellow along the margins or in the base of the V in slant cultures.

ALFALFA AGAR

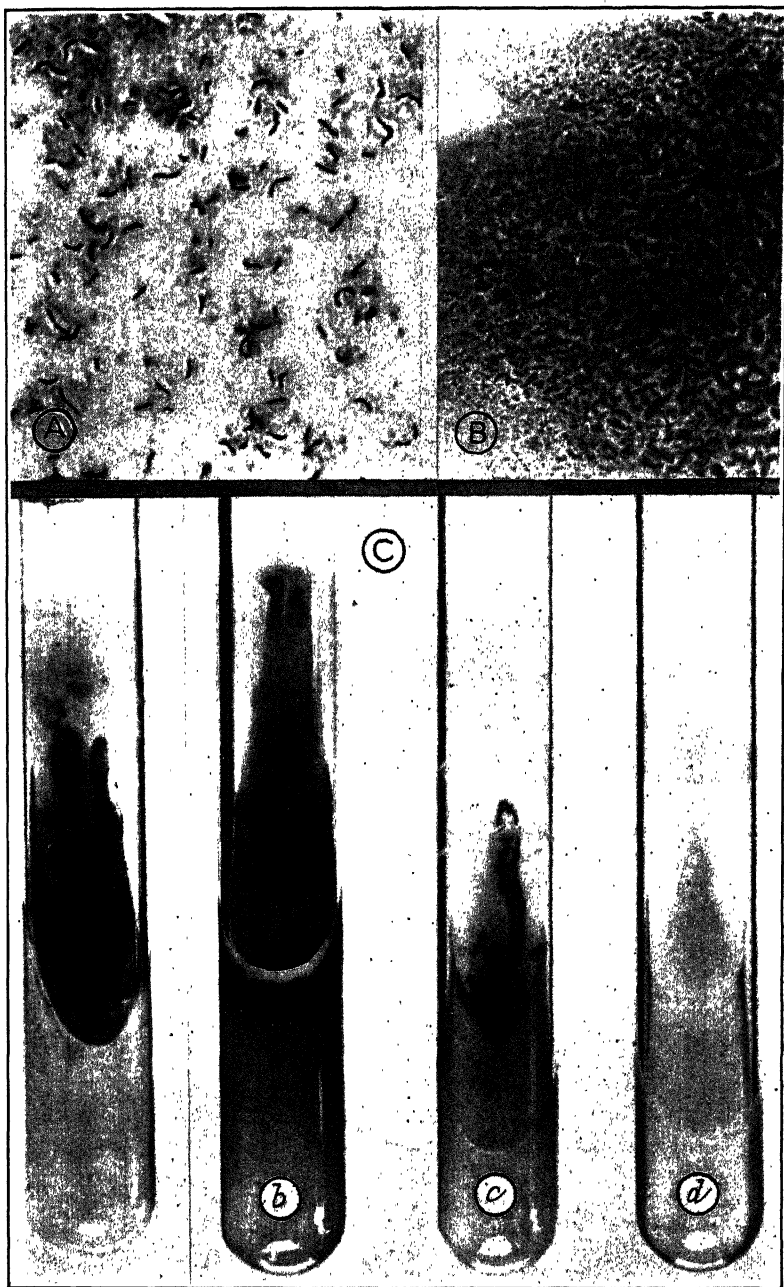
Growth is good on this medium, but not as abundant as on whey or yeast agar. Rather colorless at first, like boiled starch, becoming pale yellow. The interior is filled with small irregular cracks, producing a mottled or coarse granular effect. The surface is smooth and shining. The growth is not viscid. The medium is greened slightly more than other media containing dextrose. The blue discoloration begins early and gives a blue or green-gray color to the growth.

LACTOSE AGAR

On a medium composed of 2 per cent lactose, 1 per cent Difco peptone, and 1 per cent agar, there is a fairly good growth, which in four to six days has considerable blue and occasionally some violet color. Agar with lactose or peptone alone failed to give more than a very scanty white growth. One reason for the scanty growth in the lactose medium may be the fact that as there is no buffer present it has not been possible to keep the medium at a P_H value favorable for the organism.

BEEF BOUILLON

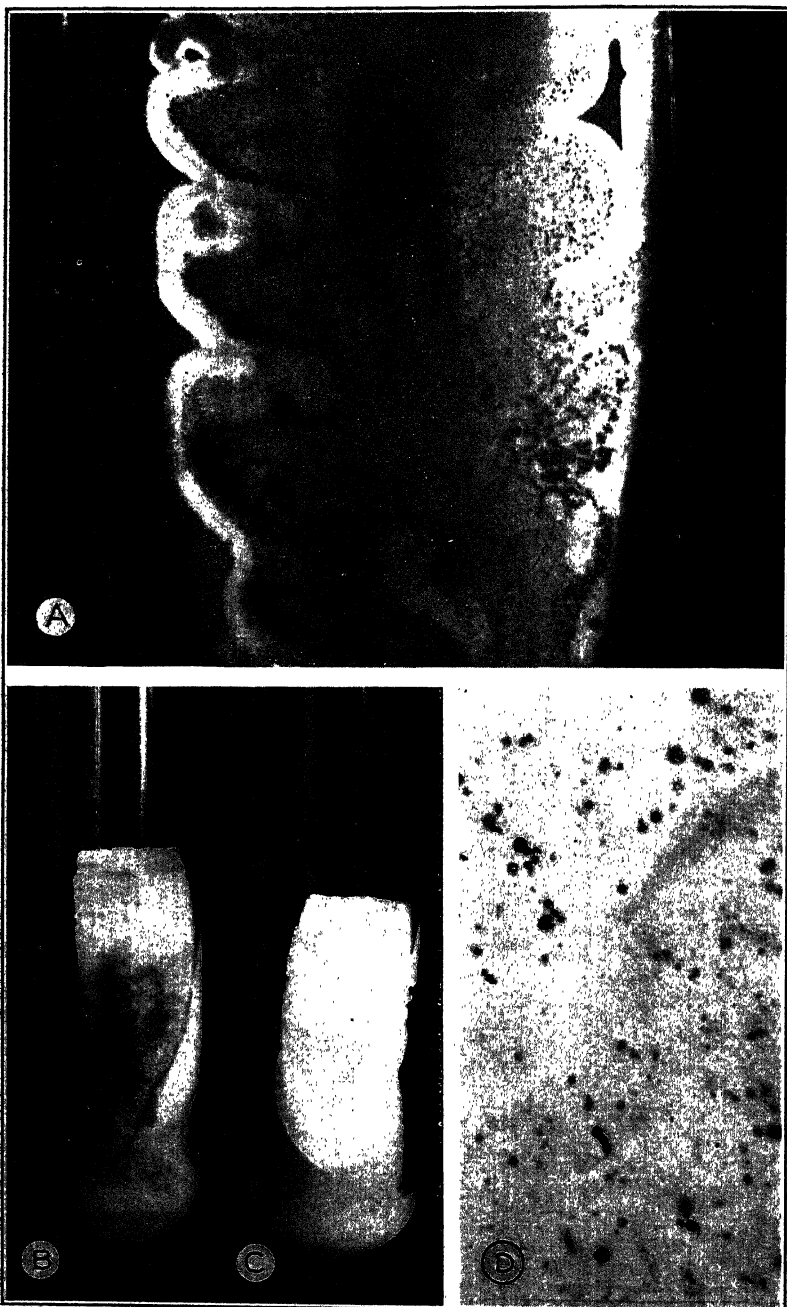
Beef bouillon of a favorable P_H value (6.8 to 7.0) rather heavily inoculated from a vigorous culture will cloud at a favorable temperature (22° to 24° C.) in 24 hours. If the medium or the temperature is even slightly unfavorable, it is not unusual for the clouding to be delayed until the third day or even later. The clouding, which never becomes heavy, is usually uniformly distributed throughout



A. *Aplanobacter insidiosum* grown on beef agar for 24 hours. Stained with Ribberts' capsule stain. \times about 1,200

B. *Pseudozoogloea* in yeast-agar culture of *Aplanobacter insidiosum*. \times 6

C. Blue and gray-blue color developed in cultures of *Aplanobacter insidiosum* on whey agar (a), bean agar (b), and potato-dextrose agar (c), in contrast with absence of color on beef agar (d)



A. Potato-dextrose agar slant culture of *Aplanobacter insidiosum*. Opaque blue granules have developed in the bacterial growth. $\times 4\frac{1}{2}$.

B. Potato-cylinder culture 10 days old grown at 18 to 20° C. Numerous blue granules and a good amount of growth.

C. Parallel with B but grown at 23 to 25°. Growth is scanty, and there are no blue granules.

Blue granules from a culture of *Aplanobacter insidiosum*. $\times 500$.

the broth, but if cultures under unfavorable conditions are not disturbed for some days the clouding often begins in a restricted area either against the wall or near the surface. The clouding may be considerable in these areas, while other parts of the medium remain clear until the tube is disturbed. This restricted character of growth seems to indicate a nonmotile organism. No pellicles are formed, and no rims except thin, irregular ones on cultures at low temperatures (2° to 8° C.) after eight weeks of growth. (Cultures in beef bouillon plus dextrose readily form thin, white rims.) The sediment is scanty, viscid, and slightly yellowish. After eight to ten weeks at room temperature the bouillon is usually clear. At low temperatures (2° to 8° C.) clouding persists for at least four months.

BEEF-GELATIN PLATES

On plates held at 20° C. tiny points of growth become visible on the fourth or fifth day. These grow slowly, requiring 12 to 25 days to reach the maximum of 3 millimeters in diameter for well-isolated colonies. The growth is at first very pale yellow and opaque; when old it is Naples yellow and transparent. Under a lens or microscope the margins are finely serrate or toothed. By the tenth day the well-isolated colonies are sunken in a shallow depression, but there is no liquid evident until about the fifteenth to eighteenth day, when the largest colonies are surrounded by depressions 7 to 8 millimeters in diameter. The depressions are shallow, entirely free from clouding and have smooth margins. A few of the larger depressions show a mere trace of liquid. After this time (10 to 15 days) both growth and liquefaction apparently cease, as there is no change, and the medium finally becomes dry. Thickly sown plates show some softening of the gelatin when about 2 weeks old. When 4 weeks old the gelatin flows only slowly when plates are put on edge.

GELATIN STABS

If inoculated from agar cultures the stab remains visible; if inoculated from liquid cultures it is invisible. A tiny pit forms in the surface, and this gradually spreads to the walls and deepens. In two weeks the depth of liquefied medium varies from 8 to 10 millimeters. It is cloudy, and the sediment is pale-yellow; no surface pellicle or rim. After three months cultures held at 18° to 23° C. are not entirely liquefied.

POTATO CYLINDERS

There is considerable variation in the potato-cylinder cultures, some of which is due to differences in moisture, in temperature, and in the potatoes, and some doubtless to the reaction of different colonies. In general, at room temperatures, the potato does not discolor (pl. 6, C) and growth becomes visible about the third day after inoculation, as pale yellow, shining areas. This growth spreads over a considerable part of the cylinder and becomes moderately thick, smooth, shining, and usually Naples yellow in color. If there is a large amount of water the growth is thinner, wet, and deeper yellow. However, there are exceptions in which both potato and growth discolor and follow the same course as cultures kept at lower temperatures.

At a temperature of about 16° C. growth begins as early, becomes more abundant, and continues longer than in cultures grown at room temperatures. The potato may not discolor but usually there are areas of blue, blue-green, or lavender over parts not occupied by visible growth. These colors may remain, but more often they disappear, change to dull-gray or blue, or are overgrown by the spread of the bacteria. The rather abundant yellow bacterial growth is so quickly and completely filled with tiny blue particles that the yellow color is visible only in the advancing margin of the most recent growth. This mixture of yellow and blue produces a variety of shades of green, from light, yellow-green to dark-green. During the period of most vigorous growth the predominating colors are "citrine," "buff-citrine," and "olive-green." Some areas may be distinctly lavender, violet, blue, or blue-green. Sometimes one color predominates, and sometimes several or all the colors may be found in one culture. As the cultures become older and drier the blue increases, and eventually the growth is a dark-blue, almost black. A trace of yellow remains for a time in the base of the tube, but this too disappears when all the water has evaporated. The discoloration does not invade the potato tissue.

In parallel cultures, tubes with a scanty supply of moisture produced earlier growth and discoloration than tubes containing a normal amount of water. But in the moist tubes growth continued for a longer period, and in the end the amount of discoloration equalled or surpassed that in the drier cultures.

A number of potato cylinders were made from a single large tuber. These gave comparatively uniform results in amount of growth and discoloration.

This discoloration of potato cylinders has occurred only occasionally at 23° to 25° C. and has not been seen at all in higher temperatures. Cultures grown at temperatures from 20° to 2° C. always became more or less discolored.

ALFALFA ROOTS

Pieces of alfalfa root were sterilized and used as a culture medium. On these the growth was scanty and of no special character. Some areas of the root, usually just above the water level, became blue-green in color and the water clouded. On the eighteenth day all the water was poured out of the tubes but no better growth resulted from this treatment. Three weeks later some of the cultures had on the wall of the tube small colonylike growths of pale yellow and many of these had bright blue centers. In a microscopic examination these blue areas were found to be composed of blue bacteria.

MILK

Five days after inoculation (at 22° to 25° C.) a pale-yellow color appears on the surface. This slowly increases to a depth of 3 to 5 millimeters and deepens in color to between "cream-buff" and "chamois." Ten to twelve days after inoculation there is a shallow layer of whey on the surface, but no noticeable curd until the eighteenth day. The whey is never very abundant, but is very clear and bright yellow (antimony-yellow is nearest color). The curd remains soft, uniform in texture, "light-buff" in color. There is no indication of

digestion of the curd in three months. The most striking character in the milk cultures is the development of color in the sediment left on the walls of the tubes by evaporation. This is at first (when cultures are 12 to 14 days old) a band of "apricot" yellow. A few days later the color is duller, and with a lens there are seen, mixed in with the yellow, numerous flecks of bright blue and lavender. These flecks of color gradually increase and some of them evidently become darker, for later the general color of the band is dark gray-blue or slate-blue, which, under the lens, shows a general mixture of bright blue, violet, gray, and greenish shades. These colors persist for at least three months. Sometimes the whole culture became pale blue-green in color on the third or fourth day after inoculation at 22° to 25° C., and earlier at 17° to 20°. This color was scarcely noticeable at the higher temperatures but was very definite at the lower temperatures. It persisted for only about 24 hours. At 17° to 20° the blue discoloration in the rims begins earlier than at 22° to 25°, and often extends over the surface of the culture, but the curdling of the milk does not seem to be hastened. Tyrosin crystals are abundant in old milk cultures.

LITMUS MILK

In four to five days at 22° to 25° C. the first change occurs, this change being the appearance of yellow in the surface layers and a trace of reduction. Reduction is complete in 8 to 10 days. No reddening at any time. After five weeks the litmus color begins to return slowly. In the evaporation band bright colors develop as in the plain milk cultures.

METHYLENE-BLUE MILK

Reduction depends, evidently, on the vigor of the inoculating material. In some tests complete reduction occurred in 24 hours, and in others it was delayed and was not complete until the eighth to tenth day. After three months the whey is pale greenish-yellow, or in tubes where the whey has evaporated there is a thin layer of greenish-blue on the surface of the cream-colored curd.

BLOOD SERUM

Stroke cultures on Loeffler's blood serum at 22° to 24° C. produce a moderate amount of pale yellow growth, slightly rough on the surface. There is no liquefaction or clearing. The medium becomes pale cream color.

STARCH AGAR

There is a moderate amount of diastasic action on starch suspended in beef agar and on potato cylinders. Tests were made with iodine potassium iodide.

CONGO-RED AGAR

Stab culture gave a slow and scanty growth of dull reddish color in which blue granules were found in moderate numbers. The color of the medium was not affected.

FERMENTATION TUBES

The culture medium was a 2 per cent Difco peptone with 1 per cent, respectively, of each of the following carbon compounds: Dextrose, lactose, sucrose, and glycerin. A moderate amount of clouding developed in five days in the open ends. The amount of growth was equally good in all of the cultures. No gas was formed. Clouding remained definitely limited to the open end. Titrations made on the fifth, tenth, and sixteenth days showed acid production increasing in all for the first 10 days. After the tenth day there was a continued increase in acid in the dextrose, sucrose, and glycerin, but in the lactose a return to the original P_H value.

These cultures gave negative results when tested for ammonia.

Sterile milk in fermentation tubes did not produce gas. The milk in the open ends reacted as other milk cultures. In the closed end the milk remained unchanged for at least two months.

Nitrate bouillon in fermentation tubes clouded moderately well in the open ends. The closed ends remained clear. No gas was produced. Tests for ammonia and for nitrites were negative.

FERMENTATION OF CARBOHYDRATES

Brom-cresol purple was used instead of litmus, as the indicator in beef agar plus 1 per cent, respectively, of the following: Dextrose, lactose, sucrose, galactose, and glycerin. The lactose agar showed only slight indications of acid formation, and this soon disappeared. In all the others the acid formation was evident in three to four days and was stronger than in lactose. In the dextrose, sucrose, and galactose the greenish-yellow color indicating acid disappeared in two to three weeks, but in the glycerin this color had not disappeared in three months. Growth was abundant on all these cultures. One of the six colonies (2021 Wis.) used in the tests always gave a stronger and longer continued acid development than the others.

REDUCTION OF NITRATES

Cultures in nitrate bouillon and in nitrate bouillon plus sugar gave good growth. Tests for nitrite were made according to the method recommended in the Manual of Methods (13). No trace of reduction occurred in any of the several experiments. Tests for ammonia were negative.

INDOL

Tests were made on cultures in Dunham's solution, peptonized Uschinsky's solution, and in a medium containing peptone, disodium phosphate, and magnesium sulphate. None of these produced any indol. *Bacillus coli* grown in the same media gave good indol reactions.

AEROBISM

The alfalfa bacteria do not grow if deprived of oxygen. Yeast, whey, and beef agars known to be favorable for growth were inoculated, some as "shake" cultures and others heavily inoculated on the surface, which was then covered by thick layers of agar. No growth occurred except on the surface.

Stabs remain visible if the inoculation is heavy and from agar cultures, and in some cases there may be slight growth in the upper part of these stabs. Stab inoculations from liquid cultures are not visible.

No clouding occurs in the closed arms of tubes containing dextrose, sucrose, lactose, or glycerin.

TOLERATION OF SODIUM CHLORIDE

A medium of peptone-beef bouillon of P_H value 6.8, and containing, respectively, 2, 3, 4, and 5 per cent of chemically pure sodium chloride was inoculated from 6-day-old beef-bouillon cultures. After four days the 2 and the 3 per cent salt bouillons showed clouding, which increased until the cultures were clouded as well as the plain bouillon controls. Examinations were made for chains but none were found. No growth occurred in the 4 or the 5 per cent salt bouillons.

TOLERATION OF ACID AND ALKALI

This organism grows best in media having a P_H value of 6.8 to 7. In peptonized beef bouillon with sodium hydroxide as the alkali and hydrochloric as the acid, the optimum is about 6.8 (+14 Fuller's scale). Its limits of toleration are P_H 5.6 (+26 Fuller's scale) on the acid side, and 8.2-8.4 (0 to -2 Fuller's scale) on the alkali side.

AMMONIA

Cultures grown in beef bouillon, nitrate bouillon, yeast agar, milk, whey agar, and various other media of various ages, gave negative results in ammonia tests made with Nessler's solution.

HYDROGEN SULPHIDE

This organism does not produce hydrogen sulphide. Lead-acetate agar made with beef infusion and with a P_H value of 6.8 was used in the tests, as both the beef extract and the P_H 7.6 recommended in the Manual of Methods (13) are unfavorable for this organism. Other tests were made by suspending lead-acetate paper over cultures in various media.

USCHINSKY'S SOLUTION

In three out of four experiments this medium showed a very faint milky color in six to eight days after inoculation. This clouding (?) did not increase. Probably a slight growth occurs in this medium if all the conditions for growth are favorable.

FERMI'S SOLUTION

In four experiments there was no sign of growth, except a slight clouding from one colony (2021 Wis.) in one experiment.

COHN'S SOLUTION

No trace of growth.

PIGMENT FORMATION

The color of the alfalfa bacteria on solid media is pale yellow (Naples yellow). In quite young cultures or in thin layers it is white or almost colorless. When grown at lower temperatures (2° to 20° C.) the color is somewhat deeper and has a tinge of orange (apricot to antimony-yellow). This deepening of color is particularly noticeable on potato cylinders and on agar containing sugar.

On most media there appear in this yellow growth, after a variable length of time, areas of darker colors, sometimes as small isolated streaks or spots, sometimes as fine specks generally distributed, which may remain restricted and make little or no increase. More often the darker color is rather evenly distributed in a considerable part of the growth, changing it to a bluish or greenish gray, often quite dark in tone, the normal yellow color remaining only in the margins of most recent growth (pl. 6, A). Examination with a hand lens shows the presence of numerous small dark particles in the growth, and also bright blue and violet colors that are not always plainly visible to the naked eye. The colors and their intensity vary somewhat in different isolations, in different media, and at different temperatures. At room temperatures the amount of discoloration is only moderate, except in some media very favorable for its development. At higher temperatures it has been observed only very rarely. At temperatures from 2° to 20° C. (from 15° to 17° seems most favorable) the discoloration increases until it entirely, or in large areas, replaces the original yellow color of the growth.

On potato cylinders there is considerable diversity in the color produced. Some of the variation is due to the difference in potato tubers. When a number of cylinders were made from one large tuber the results were more uniform. In general, there is first, before the growth is well established, a green or blue discoloration that seems to be due to a change in the potato rather than to bacterial growth. This color usually fades or sometimes is later covered by bacterial growth. The rather thick layer of growth is very quickly changed to various shades of yellow-green, yellow-brown, blue, or violet, or a mixture of all these colors, with the normal yellow color only at the extreme margins. Even these margins are eventually invaded, and the final result is a dark blue, almost black, growth. The interior of the potato is not discolored.

On potato agar and on various nutrient agars with sugar added, and on agar containing only 1 per cent peptone and 2 per cent lactose, the same colors develop as on potato cylinders, but there is less olive-green and more bright blue and violet. On agar that contain only peptone or only lactose, the growth is very scanty and no blue color develops. On agar containing 1 per cent peptone and 1.5 per cent lactic acid (P_H 7.0), the scanty, white growth had no trace of blue. In plate colonies and in streak cultures in tubes the blue usually occupies the central part with a surrounding band or line of violet. The extreme margin of growth on agar is not changed until after growth ceases, and sometimes it never has the blue color. The colors remain very bright as long as the cultures are moist. The blues vary from a strong bright blue to dark blue, and the violet ranges from pale lavender to dark purple. In tube cultures the whole surface of growth often is uniformly dark blue in color.

The surface is usually smooth, and often has a brilliant iridescence of copper, bronze, or red-gold color. As the media dries the colors become duller, but even in cultures several months old there are isolated areas of very bright color. The violet often disappears, sometimes changing to green.

Plate cultures with a restricted supply of food and moisture usually show the discoloration somewhat earlier than tube cultures.

Cultures on the usual nutrient beef agar only rarely develop a trace of blue or blue-gray. A few tube cultures and a few plate colonies have had tiny blue spots, too small to be seen without a lens.

Milk cultures have on the walls of the tubes a wide band composed of particles left there by evaporation. This material seems too far away from the surface of the culture to be affected, but evidently there is some bacterial action in it, for this band (which in check tubes remains dull white) becomes dark gray-blue. A hand lens shows that this colored band is composed of numerous small mosaic-like bits of bright blue, violet, and gray. The milk which eventually curds does not develop any of these colors. Litmus milk cultures have a similar band on the tube wall, but its change in color is less striking as it is always more or less obscured by the litmus.

Cultures in beef bouillon plus dextrose, lactose, sucrose, or galactose, form white rims on the tube wall. In the rim of the lactose medium, blue and violet colors develop, but only in slight amount. No color has been found in the bouillon cultures containing dextrose, sucrose, or galactose.

A microscopic examination of the discolored growth in all these cultures shows that the color is due to the presence of great numbers of small, opaque, blue bodies, mostly spherical but some oval. These vary in size from mere points to bodies $9\ \mu$ in diameter (pl. 6, D). The greater number are less than $3\ \mu$. There are masses 10 to $25\ \mu$ in diameter, but these are irregular in shape and seem to be composed of small spheres. The spheres seen singly and widely separated are very dark and opaque and not very definitely blue. When a number are massed together they have a beautiful bright blue color.

In addition to the opaque blue spheres there are smaller translucent spheres and rods of blue and violet. These latter are similar to the bacteria in size, shape, and appearance, and there seems no reason to doubt that they are bacteria. There are also great numbers of normal colorless bacteria in the mounts.

These spheres begin to develop first in isolated groups, gradually spreading out until they are rather evenly and thickly distributed in the cultures. They develop only on the surfaces exposed to the air.

Of the 40 or more colonies that have been under observation in subcultures, all have developed this pigment but not always to the same degree. Yeast, whey, alfalfa, and beef agars containing sugar, potato agar both with and without sugar, potato cylinders, and milk, are media very favorable for this color change. Of the sugars used in the media, lactose seems most favorable, with dextrose, galactose, and saccharose following in the order named. Agar containing only peptone and lactose produces the color. Sometimes the discoloration becomes visible in so short a time (two to six days) that it can

scarcely be caused by old age. Usually the change does not occur until the growth has reached and passed its maximum, and it is more likely to begin in the oldest center of growth. Transfers made from various parts of the same culture show that bacteria taken from the discolored areas are very much slower in growth than bacteria taken from the normal yellow areas. Often they do not produce any growth.

Exposure to direct sunlight has no noticeable effect on the pigment. Cultures, also thin layers of the blue granules spread on glass, were kept in a south window for a number of days, but no change in color occurred, and a microscopic examination showed the blue granules and smaller violet bodies to be unchanged in appearance.

When the blue growth was mixed with water the water became pale blue. In a corked vial this color persisted for six weeks. The addition of an equal amount of 4 per cent NaOH did not affect the color. A small amount of H_2SO_4 caused the color to fade entirely. After being in water six weeks the blue granules seemed unchanged.

Cold water does not dissolve the granules.

Heated over an open flame in water, no effect.

Heated to dryness over flame, no effect.

Boiled in water in test tube for five minutes, no effect.

In 95 per cent alcohol; not dissolved and alcohol not blued.

In 50 per cent alcohol plus a small amount of NaOH, heated, all the granules disappeared.

In 50 per cent H_2SO_4 the granules dissolve, leaving a violet stain, which later disappears.

In 3 per cent HCl; no change.

In glacial acetic acid; no change.

In 10 per cent NaOH the granules slowly dissolve; 5 to 10 minutes are required for the disappearance of average-sized granules; the action is quiet and no trace of color remains.

Mellon's reagent has no visible effect on the granules.

TEMPERATURE RELATIONS

The maximum temperature for growth is $31^\circ C.$ for some isolations, and 28° to 30° for others. The minimum is somewhere below freezing, and the optimum between 21° and 24° . There is considerable variation in different isolations and in different media. Of the different isolations tested only two grow at 31° (colonies 2021 and 2027 isolated by the senior writer at Madison, Wis.), and these usually only on solid media at this temperature, the growth becoming visible about the sixth day. Occasionally a beef-bouillon or Dunham's-solution culture of these two colonies clouds at $31^\circ C.$

The other isolations grow at 28° to $30^\circ C.$ on solid media, forming tiny, isolated colonies on the slants in 8 to 10 days. Beef-bouillon cultures seldom clouded, even at 27° . In these experiments the beef bouillon and the beef agar were equal in P_H values, in amount and kind of inoculation, and were in the same incubator at the same time. Dunham's solution clouds more readily than beef bouillon, and some of this group produced growth in it at 29° ; while parallel cultures in beef bouillon produced no growth. Parallel cultures of these were made on beef agar and in beef bouillon and kept at 31° for 15 days. Not a trace of growth occurred. The cultures were then left at

room temperature where all the agar cultures later produced normal growth, but only one bouillon culture clouded.

In parallel cultures of beef bouillon and of Dunham's solution, adjusted to the same P_H , the Dunham clouds somewhat earlier than the beef bouillon, at room and higher temperatures. At lower temperatures there is less difference in the time of clouding. The optimum and the maximum temperatures are higher in Dunham's solution than in beef broth of the same P_H value.

The organism very plainly does not like temperatures above 27°C ., but if growth does start it is able to continue and eventually becomes fair in amount. Some cultures on whey and yeast agar at 27° to 28° have had, after 15 days, growth equal in amount to cultures grown at 22° to 25° .

These maximum temperatures for growth have been with media having a P_H value known to be favorable for growth, and with rather heavy inoculations from well developed, vigorous cultures. If the media is even slightly unfavorable, or the inoculum too old, too young, or too scanty in amount, no growth occurs at the maximum temperatures as stated. Beef bouillons and agars with 1 per cent dextrose and whey and yeast agars are more likely to give growth at the higher temperatures than plain beef media.

Cultures of the Alabama and Mississippi strains were kept at 26° to 31°C . for 45 days. No growth occurred. Another lot at 31° to 34° gave no growth in 80 days. Controls at 23° showed growth in two days.

The minimum temperature for growth is below 1°C . Both solid and liquid media known to be favorable for growth were kept over night at 6° to 7°C . The next morning they were inoculated quickly and in less than an hour placed in a temperature of -1.66°C . Control cultures at 28° to 25° developed good growth in five days. After 33 days the cultures at -1.66° were examined and a fair amount of growth found on the solid media (whey agar and alfalfa agar). Beef bouillon plus 1 per cent dextrose cultures had no visible growth, but all clouded within nine days after removal to 23° to 25°C .

At 1.5° to 2°C . beef bouillon and Dunham's solution cultures cloud in six to seven days. The growth continues and eventually is as good as at the optimum temperature. Cultures on beef agar and potato cylinders also grow well at 1.5° to 2° . The beef-agar cultures had after three months heavier growth than cultures ever produce at room temperatures. The retention of moisture doubtless contributes to continued growth.

Up to the present it has not been possible to determine exactly the optimum temperature for growth, as this optimum lies between the ranges of the available cold chambers and incubators. Room temperatures do not remain constant long enough for good comparisons with this slow-growing organism. Parallel sets of cultures were grown in four different locations with temperatures of 16° to 17° , 19° to 20° , 22° to 23° , and 23° to 26°C . Often when growth was unusually slow in starting all of these would show clouding at the same time, and occasionally clouding showed first at 19° to 20° , or even at 16° to 17° . When the growth was more normal, clouding was visible first in the cultures at 22° to 23° , and these continued to have best growth for several days, although the others usually clouded very soon after. From about the fourth to the tenth day after growth

begins, the cultures at 19° to 20° and 16° to 17° are more heavily clouded than those at higher temperatures. After this, however, all are equal in amount of clouding. The 23° to 26° temperatures seemed always less favorable than the 22° to 23°.

Cultures on solid media have given about the same results as the broth cultures. Growth continues longer and eventually becomes more abundant at the lower temperatures, probably because the media does not become dry so readily.

VITALITY

FREEZING

The freezing of freshly-inoculated beef bouillon cultures for two hours caused a slight reduction in the number of viable bacteria. Sixteen hours' freezing killed all the bacteria in one isolation (2001), and 89 per cent in another (15). In another experiment two hours freezing caused a slight reduction, and 24 hours an 89 to 93 per cent reduction.

Beef-bouillon cultures 3 days old were frozen for 16 hours without causing any noticeable reduction in the number of viable bacteria, but they were slower in growth than before freezing.

SUNLIGHT

This organism is not easily killed by exposure to direct sunlight. Four experiments indicate that one hour's exposure kills only a small proportion. But the colonies were slow in appearing. Exposures of one and one-half, two, and three hours either entirely killed or greatly reduced the vitality of all the organisms, on the exposed side of the plate. Occasional colonies appeared even after two hours, but none after three hours exposure. In all the sunlight tests there was observed a slow advance of growth in the form of tiny colonies, from the protected side of the plate into the medium on the exposed side. This sort of growth advanced sometimes as much as 10 millimeters. All the sunlight tests were made with the cover on the poured plates. The plates were placed so that the sun's rays were perpendicular to them.

IN CULTURE MEDIA

This organism has been under the junior writer's observation only seven months, too short a time to learn its limits of vitality on artificial media. Five-month-old cultures grown and kept at room temperature are still alive, but are slow in renewing growth when transferred. Cultures grown and held at lower temperatures, 2° to 10° C., for five months seem as vigorous as young cultures. There seems to be no difference in the vigor of cultures on liquid and solid media.

DESICCATION

To date the extreme limit of vitality after drying on cover glasses is 23 days. The greater number have shown no growth after drying for five days.

LOW TEMPERATURE

At 1.5° to 2° C. growth is visible in liquid media in 7 days, and on solid media in 15 days. In one month or less, visible growth develops at -1.6° C. (29° F.).

HIGH TEMPERATURES

Beef-bouillon cultures kept for 10 minutes in a water bath at 52° C. were killed. Beef-bouillon cultures kept at 35° to 36° for 10 days were killed.

Some beef-agar cultures held at temperatures of 28° to 32° C. for 23 days produced a few very tiny colonies on the slants. When removed to a temperature of 22° to 25° the growth increased, in spite of the rather dry surface.

Some potato-cylinder cultures at 28° C. for 47 days produced a scanty amount of growth. When removed to a temperature of 22° to 25° growth became nearly normal.

The following cultures made no growth at the temperatures given, but were not killed as was evident from the growth that developed after the cultures were removed to 22° to 25° C.:

Beef bouillon, 28° to 32° for 23 days.

Beef agar + 1 per cent dextrose, 30° to 32° for 14 days.

Potato agar + 1 per cent dextrose, 30° to 32° for 14 days.

Potato cylinders, 30° to 32° for 14 days.

Potato cylinders, at 28° for 24 days.

Potato cylinders, 33° to 37° for 17 days.

Whey agar, 29° to 30° for 11 days.

Whey agar, 31° to 33° for 11 days.

Some beef-bouillon cultures held at 35° to 36° C. for three days were then removed to 24°. Growth appeared first in these cultures 9 to 16 days after removal to the lower temperature.

Some beef-bouillon cultures held at 35° to 37° C. for five days and then removed to 24° never produced growth.

Whey-agar slant cultures kept at 30° to 32° C. for seven weeks did not grow. These were then removed to a temperature of 22° to 23° C. but no growth had developed eight weeks later.

Some beef-bouillon cultures were also at 30° to 32° C. for seven weeks, then at 22° to 23° for eight weeks. No growth developed.

TECHNICAL DESCRIPTION

APLANOBACTER INSIDIOSUM L. McC. (6)⁷

A nonmotile rod with rounded ends; single or in pairs, no chains have been found; single rods 0.7 to 1.0 × 0.4 to 0.5μ; capsules present; no spores; gram-positive; not acid fast. On peptone-beef agar the colonies are round, smooth, shining, flat to slightly raised, amorphous, white becoming pale yellow; in beef broth, moderate, uniform clouding without rim or pellicle; trace of growth occurs in Uschinsky's solution; no growth in Fermi's or Cohn's solution; gelatin slowly liquefied; blood serum not liquefied or cleared; optimum temperature about 23° C., maximum 28° to 31°, minimum below 1°, thermal death point 51° to 52°; no indol, ammonia, or hydrogen sulphide produced; aerobic; moderate diastasic action; soft curd forms in milk in 18 to 20 days; casein is not digested; blue and violet pigments develop in the evaporation band on wall of milk cultures; growth yellow on most culture media, but on potato cylinders and on agar containing sugar, opaque dark-

⁷ See footnote 4, p. 502.

blue granules develop in the growth in sufficient numbers to change the color to various shades of blue and green; litmus reduction complete in 8 to 10 days; nitrates are not reduced; acid without gas produced from sucrose, dextrose, lactose, galactose, and glycerin; pathogenic on *Medicago sativa*, filling the vascular system and causing death of the plants.

According to the descriptive chart of 1920 indorsed by the Society of American Bacteriologists (13), its group number is 5331-31135-1222.

SUMMARY

The most serious and threatening disease of alfalfa in some irrigated districts and the more humid areas of the United States appears, from a study conducted largely in 1925, to be the bacterial wilt and root rot. The causal organism (*Aplanobacter insidiosum* L. McC.) is described fully (for the first time) in this paper.

The disease is chiefly vascular in character, the bacteria passing from the taproot to each succeeding weakened crop of stems until the plant is killed. The most conspicuous symptom is a dwarfing of severely diseased plants and pale color of foliage, and small, narrow leaflets yellowed and curled at the margins. The taproot when cut shows yellow or brown discoloration beneath the bark.

The disease is now known in many localities from New Jersey to Idaho and south to Mississippi.

Artificial inoculation of alfalfa plants has been accomplished only through wounds, introducing the parasite directly into the vascular system through cut stems, or into cortical tissue of roots whence it makes its way into vessels. In either case it enters the outermost vessels of the taproot, which are soon filled with a pale yellow mass, becoming deeper in color and more dense and gummy in consistency with age. This vascular invasion may extend to the veins of the leaves, but has not yet been demonstrated in seed.

The bacteria make some progress in filling vessels in the taproot at a temperature as low as 9° C. Symptoms of disease and the death of infected plants appear to be hastened by retardation of root growth brought about by high soil temperature or frequent cutting of tops.

This parasite has been found producing a wilt disease in *Melilotus alba*.

Control measures are suggested.

The bacteria have been isolated from fresh roots and stems, also from stems kept in a dry atmosphere for five months.

In culture media the organism grows rather slowly. Isolation plates seldom show growth in less than five days. Potato or some sugar in the medium encourages growth.

In culture media the bacteria grow best at about 23° C., but they also grow well at lower temperatures. Growth is able to begin and to continue at 1°. Growth seldom begins at 31°, but once started it is able to make some progress.

On solid media the growth is yellow, but on most media this yellow color is soon obscured by the formation in the growth of numerous small blue particles which change the color to various blues and greens, blues predominating.

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BUD SPORTS IN SWEET POTATOES ¹

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In the fall of 1923, in a planting of the Haiti variety of sweet potato (*Ipomoea batatas* Poir) in Delaware, one plant was found which produced three red and three yellow roots, as shown in Plate 1. The Haiti is normally a red variety, marked with small oval or elongated whitish to yellow spots which vary somewhat in size. (pl. 1).

No information seems available as to the origin of the Haiti variety—that is, whether it originated as a bud sport, a mutation, or as a result of hybridization. In the section of the United States in which this plant was found, flowers are rarely produced and seeds never, the sweet potatoes being propagated by sprouts from the roots. For a period of 12 years before the specimen shown in the illustration was collected this particular strain of Haiti is known to have been propagated by sprouting of the roots. It therefore in all probability is not the result of hybridization. Inasmuch as sweet potatoes are propagated by the production of sprouts from the roots, it is practically equivalent to the perpetuation of the same plant over an indefinite period of years.

When photographic and painting records of the roots had been made, the reds and yellows were segregated and bedded and the progeny followed over a period of two years. The three red and three yellow roots were bedded in the greenhouse in the fall of 1923, and they gave in all about 25 plants of each strain. These plants were set in the field in the spring of 1924, and about one peck of potatoes were produced from each strain. The reds produced all reds, and the yellows all yellows. In the spring of 1925 all the roots of both lots were bedded, and several hundred plants of each were produced. Instead of setting out all these plants, 100 were pulled in such a manner as to obtain 2 or 3 from each potato. These were planted and cultivated in the customary way and harvested in the fall. Nearly 2 bushels of each color were obtained, the reds producing only reds and the yellows only yellows.

Figure 1, a photograph of the progenies, shows the general similarity in size and shape between the reds and yellows, there being no greater difference than would ordinarily be found between two lots of the same strain. This observation is interesting in view of the fact that the yellow potatoes of the original hill (pl. 1) are long and more slender and would ordinarily be classed as rooty. The potatoes of the Haiti variety are normally large and rather chunky. These results, therefore, would seem to indicate that the progeny tend to revert to the characteristics of size and shape of the parent stock, rather than to inherit those of a single root from an individual hill.

¹ Received for publication Dec. 19, 1925; issued September, 1926.

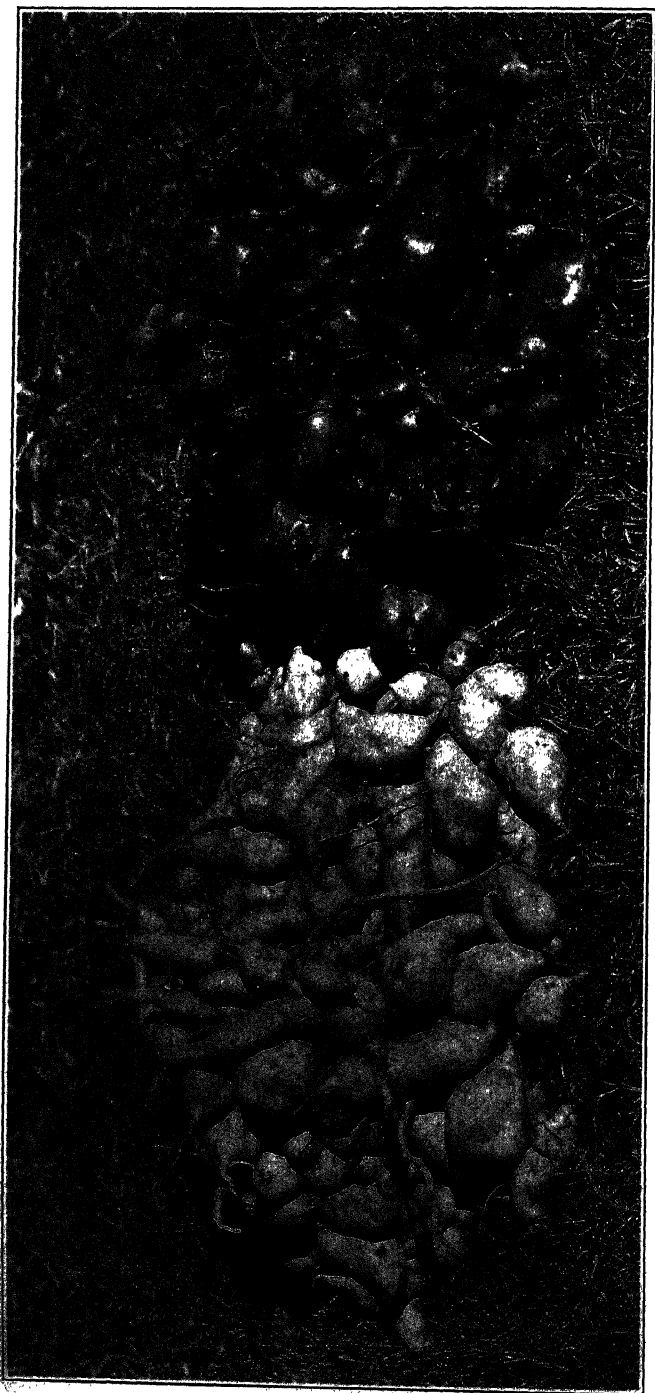


FIG. 1.—Progenies of the two types segregated from the plant of Haiti variety of sweet potato shown in Plate 1, showing general similarity in size and shape between the red and yellow potatoes. Propagation of the two strains was carried on for two consecutive years, the yellows (left) producing yellows, and the reds (right) producing reds.



Showing color, shape, size and arrangement of dark and yellow patches on the stem of the original plant and plant grown from seedling. The yellow patches are more or less round, irregularly shaped and often of a size as shown. (This is from a colored photograph). Potatoes were grown under glass and taken from under the covers.

The color of the stem and vines of the Haiti variety is a deep purple. This color extends also to the petioles. In the yellow strain some of the purple pigment was lost. As a matter of fact, it has been determined from the color of the stem and vines whether a plant bore yellow or red potatoes. There has been no apparent difference in the shape, size, or color markings of the leaves, or in the time of maturity or vigor of the plants of the two strains.

THE RELATION OF HUMIDITY TO INFECTION OF THE SWEET POTATO BY RHIZOPUS¹

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INTRODUCTION

Air moisture is one of the most important environmental factors in plant responses, not only as it affects the physical conditions and physiology of the plant, but because of its influence on the infection and the development of diseases caused by microorganisms. For the most part, it has been assumed that high humidity favors, and is even essential to, infection. This generalization is based largely on observation rather than on experimental data. It seems quite likely that it is generally true, but like many generalizations it is too broad and may not hold in many cases. It has been assumed, also, that within the range of humidities at which infection occurs there is a tendency for the number of infections to increase with the increase in relative humidity. There are some data to justify this conclusion, but they are not a sufficient basis for a generalization. Neither of the assumptions just mentioned holds in connection with infection of the sweet potato by *Rhizopus*.

Manns (5),² who seems to have in mind the losses due to *Rhizopus* soft rot and black rot, regards a relative humidity of 80 per cent and above as inimical to the keeping of sweet potatoes. His conclusions were based on bell-jar and storage-house experiments. The bell-jar experiments were limited in number and there was no provision made for air exchange; and in the storage-house experiments the factors, such as temperature, humidity, and wounding, were not controlled or definitely isolated, and apparently he did not study the diseases separately. The present writers were able to confirm his results only in part.

The purpose of this paper is to present the results obtained from a study of the effects of air moisture on the infection of the sweet potato by *Rhizopus* (*nigricans* and *tritici*) under certain conditions.

APPARATUS AND CONTROL OF CONDITIONS

The experiments discussed below were conducted either in the infection chambers described in an earlier publication (4), or in a sweet-potato storage house constructed according to plans designed by the United States Department of Agriculture (6).

The temperature of the infection chambers was controlled in the manner described in the above-mentioned publication (4). The control of humidity was accomplished by means of water and saturated salt solutions in evaporation pans (3). Continuous air exchange

¹ Received for publication Mar. 2, 1926; issued September, 1926.

² Reference is made by number (italic) to "Literature cited," p. 538.

was provided in each instance (4). A storage house 40 feet long, 20 feet wide, and 9 feet high, was partitioned into two rooms of the same size. In the one room a high humidity was maintained by means of a fine spray of water, the small droplets of the spray being entirely evaporated in the part of the room in which the potatoes were placed. In the other room a lower humidity was obtained by means of calcium chloride in a large evaporating pan. The temperature of the two rooms was governed by the outside temperatures, and was nearly the same in both rooms in each experiment. The difference was believed to be insufficient to be a disturbing factor.

MATERIALS USED

The Yellow Jersey, Big Stem Jersey, and Porto Rico varieties of sweet potato were employed. The species of *Rhizopus* used were *Rhizopus nigricans* Ehr. and *R. Tritici* Saito.

EXPERIMENTAL DATA

HUMIDITY AND INFECTION OF HALVED ROOTS

The infection of the sweet potato by *Rhizopus* occurs almost exclusively through fresh wounds, so in order to study the influence of humidity on infection it is necessary to work with freshly wounded roots. In a series of 15 experiments the roots were cut in halves lengthwise and placed at various humidities at a constant temperature of 23° C. In 12 of the experiments the potatoes were inoculated by dipping them in a suspension of *Rhizopus nigricans* and *R. tritici* spores; in the other 3 experiments the potatoes were not inoculated. The effects of inoculation, if any, were merely to increase the percentage of infection at the various humidities. In previous work (4) it was found that the inoculation of wounded potatoes by means of a spore suspension did not alter the temperature range of infection of either of these species, obtained when the potatoes were not inoculated. The species isolated from decayed potatoes in these present experiments was mostly *R. nigricans*, *R. tritici* being isolated only in a few instances. This fact was in accord with previous experience (4). Since the absence of inoculation did not in any way alter the relations found in connection with the inoculated potatoes, the results from inoculated and uninoculated roots will be considered together.

Table 1 gives the results of seven experiments in which the Yellow Jersey was the variety of potato used. A range of five different humidities was employed in each experiment. Wherever the same humidity was obtained during the successive experiments, the results are considered collectively. The figures in the last column represent the number of times any particular humidity was employed during the seven experiments. The percentage of infection rose from 21 per cent at humidities of 93 to 98, to 96 and 100 per cent at relative humidities of 76 to 82 per cent. At relative humidities of 48 to 53, the percentage of infection was 25 per cent. Only one experiment of this series included a relative humidity of 76, consequently the percentage of infection obtained may be high for this humidity, which will be indicated by results recorded in Table 2.

TABLE 1.—*The relation of humidity to infection of Yellow Jersey sweet potatoes by Rhizopus*

Temperature, ° C.	Depression of wet bulb, ° C.	Relative humidity	Number of halved roots used	Number of halved roots infected	Percentage infected	Time of exposure in days	Number of experiments at each humidity
23.6	0.2-0.8	93-98	170	35	21	3-11	5
23.1	1.0-1.3	90-92	496	216	44	3-11	13
23.2	1.6-1.9	84-87	98	63	64	3-11	3
23.7	2.2-2.6	79-82	308	295	96	3-11	9
23.4	3.1	70	18	18	100	11	1
22.5	6.2-6.9	48-53	120	30	25	3-9	3

TABLE 2.—*The relation of humidity to infection of Yellow Jersey sweet potatoes by Rhizopus*

Temperature, ° C.	Depression of wet bulb, ° C.	Relative humidity	Number of halved roots used	Number of halved roots infected	Percentage infected	Time of exposure in days	Number of experiments involved
23.4	0.2-0.4	97-99	90	9	10	7-11	3
23.5	0.6-0.9	93-95	240	58	24	3-12	5
23.0	1.0-1.5	87-92	696	262	38	3-15	18
23.3	1.6-1.9	84-86	178	101	57	3-11	5
23.0	2.0-2.5	80-84	388	320	82	7-11	11
23.0	2.6-2.8	78-79	120	105	88	3-12	3
22.7	3.0-3.4	72-76	138	89	64	7-15	4
22.4	4.4-5.3	59-65	200	19	10	7-15	5
22.6	6.2-6.9	48-52	160	36	23	3-12	4

In Table 2 are recorded the results given in Table 1, combined with the results of five other experiments conducted later. These latter five experiments also had to do with the Yellow Jersey variety of sweet potato. The purpose of giving the two tables is to show the variation in the results obtained from different experiments, and at the same time to show the general consistency of the data obtained.

The percentage of infection rises from 10 per cent at relative humidities of 97 to 99, to 82 and 88 per cent at relative humidities of 78 to 84, and drops to 10 per cent at relative humidities of 59 to 65, and rises to 23 per cent at relative humidities of 48 to 52. It is not known whether this last rise has any significance, although a similar relation was found in an experiment with the Porto Rico variety. Possibly if a larger number of experiments were conducted, a higher percentage of infection at relative humidities of 59 to 65 per cent or a lower percentage of infection at relative humidities of 48 to 52 might have been obtained.

In Table 3 are given the results in which the Porto Rico variety was used. The percentage of infection increased from 15 at relative humidities of 93 to 97, to 98 at relative humidities of 75 to 76. There is a slight drop in the number of infections as the humidity is lowered. At a relative humidity of 63 the percentage of infection was 81, and at a relative humidity of 52 the percentage of infection was 75. The percentage of infection at these latter humidities is high in contrast to that obtained with Yellow Jersey. It should be noted, however, that the results were obtained from one trial at each of the two humidities from two experiments. In a third experiment run late in the season, when the outside temperature was high and consequently that of the chambers (27° C.), the percentage of infection at a relative

humidity of 61 was 22. It is possible that if a larger number of experiments were conducted at these humidities the percentage of infection might be lowered. There is a close correspondence in the percentage of infection of Porto Rico to that of Yellow Jersey at humidities between 75 and 98.

TABLE 3.—*The relation of humidity to infection of Porto Rico variety of sweet potatoes by Rhizopus*

Temperature, °C.	Depression of wet bulb, °C.	Relative humidity	Number of halved roots used	Number of halved roots infected	Percentage infected	Time of exposure in days
25	0.4-0.8	93-97	54	8	15	6-7
24	1.2-1.6	86-91	110	41	37	6-7
23.5	3.1-3.2	75-76	56	55	98	6-7
22.0	4.8	63	32	26	81	7
22.6	6.2	52	24	18	75	6

Figure 1 represents graphically the results recorded in Tables 1, 2, and 3. The percentage of infection is plotted against the depression of the wet bulb, because these figures are more exact than the relative humidities. The relative humidities are also given, so that the relation of infection to relative humidity can be seen at the same time. The correspondence of the three curves at relative humidities between 75 and 98 is striking. At the lower humidities there is considerable variation in the number of infections. It should be stated, however, that the higher humidities were obtained a larger number of times in each case than the lower humidities, thus tending to remove the effects of variation in the individual experiment. The values given in the three tables and in Figure 1 involve considerable variation and are not to be regarded as absolute, but relative. They represent general relations only.

The experiments conducted in the infection chambers were supplemented by two experiments run in a storage house partitioned into two rooms. In one room a high humidity was maintained and in the other a low. The Porto Rico and Big Stem Jersey varieties of sweet potato were used. The roots were not inoculated in either experiment. The results are recorded in Table 4.

TABLE 4.—*The relation of humidity to infection by Rhizopus, storage-house experiments*

Variety	Temperature, °C.	Depression of wet bulb, °C.	Relative humidity	Number of roots employed	Number of roots infected	Percentage infected	Time of exposure in days
Porto Rico.....	19.6	0.23	97	286	28	10	7
Porto Rico.....	28.0	0.53	96	115	33	29	5
Porto Rico.....	22.0	1.4	87	581	178	31	7
Porto Rico.....	29.0	2.6	82	125	108	86	5
Big Stem Jersey.....	28.0	0.53	96	173	48	28	5
Big Stem Jersey.....	29.0	2.6	82	98	65	66	5

The data are arranged in Table 4 for each variety of potatoes according to the humidities irrespective of the temperatures, which differed in the two experiments. In spite of this difference in temperature, there is an increase in the percentage of infection with the lowering of the humidities in the case of Porto Rico. The results of each experiment, however, should be considered separately. The first experiment had to do with Porto Rico, and had a duration of seven days. The high-humidity room was run at an average tem-

perature of 19.6° C., and the low humidity room at 22°. The average relative humidities of the two rooms were 97 and 87 per cent, respectively. The percentage of infection at a relative humidity of 97 was 10, in contrast to 31 at a relative humidity of 87.

In the second experiment both Porto Rico and Big Stem Jersey were used. The two rooms were run at an average temperature of

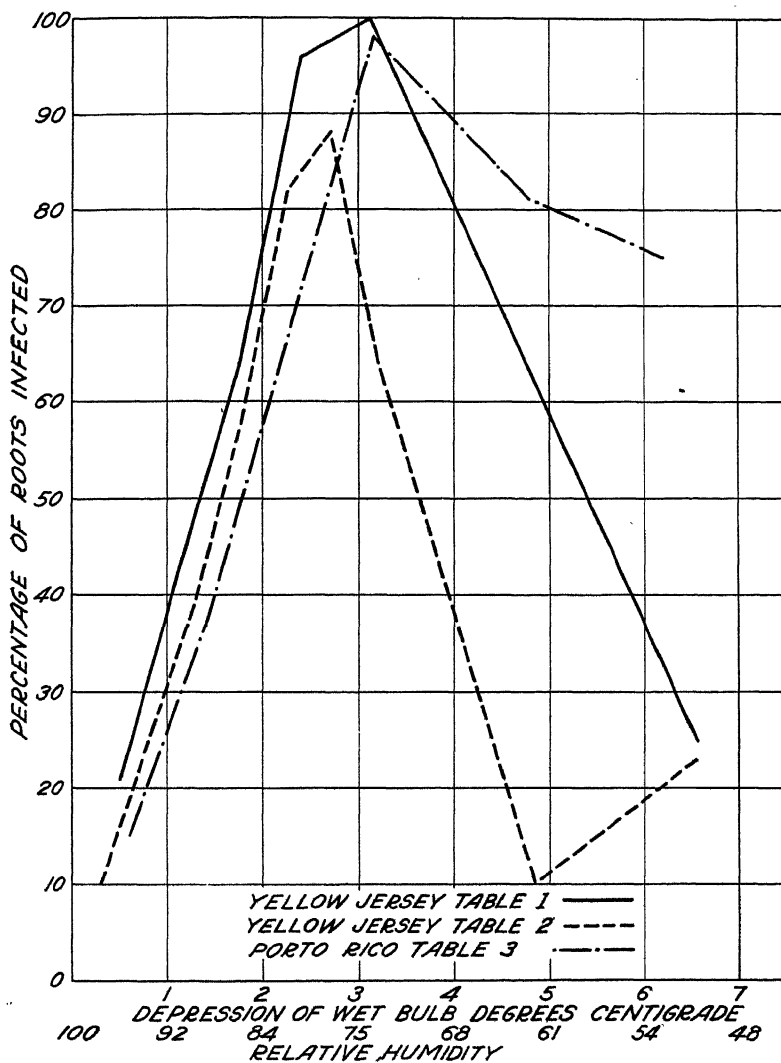


FIG. 1.—Relation of humidity to the percentage of infection of sweet potato by *Rhizopus*

28° and 29° C., and at an average relative humidity of 96 and 82 per cent, respectively. The experiment ran for five days. The percentage of infection of the Porto Rico at a relative humidity of 96 was 29, and 86 at a relative humidity of 82. At a relative humidity of 96 only 28 per cent of the Big Stem Jersey roots were infected, in contrast to 66 per cent at a relative humidity of 82.

The results of these two experiments within the range of humidities used correspond with the results obtained in the infection chambers, although there was not complete duplication of varieties. Yellow Jersey was not available at the time.

One experiment was conducted at a temperature of 12° C. The results, which will be published in another paper, correspond with those obtained at 23°, except that the range of infection was shifted nearer to 100 per cent humidity.

THE EFFECTS OF HUMIDITY ON HOSTS AND PATHOGENES

An effort was made to determine whether or not the relation of humidity to infection found in the foregoing experiments was due, on the one hand, to some reaction on the part of the host or, on the other, to a reaction on the part of the pathogenes, or to a combination of both.

A series of experiments were conducted in which halved roots were first subjected to one humidity for a time and then exposed to another for another interval of time at the same temperature (23° C.).

Very few potatoes (Table 5) which had first been subjected to high relative humidities (89 to 95) for from 4 to 12 days and then exposed to lower humidities (48 to 85 per cent) for 6 to 13 days became infected. If on the other hand halved roots were first exposed to low humidities (51 to 74) for 4 to 12 days and then placed at high relative humidities (84 to 95) for 6 to 31 days, a large number of infections resulted. For example, out of 48 potatoes which had been held at relative humidities of 89 to 91 and then removed to relative humidities of 80 to 85 there were no infections (Table 5). Out of 62 potatoes held at 89 to 95 and then removed to humidities of 48 to 73, 2 (or 3.2 per cent) were infected, while out of 182 potatoes held at humidities of 51 to 74 and then removed to humidities of 84 to 95, 123 (or 68 per cent) were infected.

TABLE 5.—*The effects of humidity on infection of halved roots exposed first to one humidity and then to another*

Temperature °C.	First depression of wet bulb, °C.	First relative humid- ity	Time of exposure in days	Second depression of wet bulb, °C.	Second relative humid- ity	Time of exposure in days	Number of halved roots used	Number in- fected	Per cent- age in- fected	Number of experi- ments at each humid- ity
23	0.4-1.3	89-97	6-9	0.5-1.3	89-95	8-31	160	9	6	7
23	1.1-1.2	90-91	9	1.9-2.2	80-85	9	32	0	0	2
23	0.6-1.3	89-95	6-12	3.3-3.6	71-73	10-13	34	0	0	2
23	0.6-1.3	89-95	9-12	6.4-6.9	48-51	12-13	28	2	7	2
23	1.3-2.4	79-89	4-12	0.5-1.2	90-95	6-31	131	12	9	6
23	1.4	89	4	2.0	84	6	16	0	0	1
23	2.1	81	12	3.3	73	13	14	0	0	1
23	3.2-3.4	73-74	6-7	0.5-1.1	91-95	8-31	11	3	27	2
23	4.8-5.3	59-63	6-7	0.5-1.1	91-95	8-31	101	79	78	3
23	6.2-6.5	54-52	6-12	1.0-1.3	89-92	9-13	29	29	100	3
23	5.5-6.3	52-57	4-9	1.9-2.0	84-85	6-9	41	12	29	3
23	6.5	51	12	3.3	73	13	10	0	0	1
23	6.3-6.5	51-52	9-12	6.4-6.9	48-51	9-13	21	0	0	2

The potatoes held at high and low humidities were examined at the time the potatoes were changed from one humidity to another. The decayed potatoes were removed from the experiment and some of the remaining potatoes were retained at these humidities while others were removed to other humidities for a certain period (Table

5). At the end of this period very few of the potatoes retained at a high humidity (80 to 97 per cent) were infected, and none of the potatoes retained at a low humidity (48 to 73 per cent) were infected (Table 5).

Figure 2A represents the effects of exposing halved roots first to a relative humidity of 59 for 7 days, then to a humidity of 93 for a period of 11 days, at a temperature of 23° C.; figure 2B represents potatoes held at a relative humidity of 93 throughout the 18 days. The contrast is striking. *Rhizopus* has grown over the cut surfaces of the potatoes first exposed to a low humidity and then placed at a high humidity, whereas the potatoes exposed to the high humidity through the entire period have remained sound. At the end of the first period (7 days) the potatoes decayed at this time were removed from the experiment, so that the results represented in Figure 2



FIG. 2.—Infection of the sweet potato by *Rhizopus*. A, Sweet potatoes held at a relative humidity of 59 per cent for 7 days, and then at a humidity of 93 per cent for 11 days; B, Sweet potatoes held at a relative humidity of 93 per cent throughout the 18 days. Temperature 23° C. in both cases

afford a comparison of the effect of high humidity on potatoes which had previously been exposed in the one instance to a high humidity and in the other to a low humidity. It is suggested from these results that some reaction occurred in the host and not in the fungus at humidities of 85 to 100, which interfered with infection, because the fungus is capable of causing infection at high humidities if the potatoes have been subjected first to low humidities. On the other hand, very few potatoes that have been exposed to high humidities become infected no matter to what humidities they are exposed later.

It is believed that the effects of air moisture on the pathogenes interfere with infection at the low humidities, since roots that have been subjected to low humidities readily become infected when removed to high ones.

THE CHARACTER OF THE RESISTANCE EXHIBITED BY THE HOST AT HIGH HUMIDITIES

Is the resistance shown by sweet potatoes to the attack of *Rhizopus* at high humidities due to a reaction of the entire root, or is it located

in the wounded surface? An effort was made to answer this question in the following experiments.

Table 6 contains the results of two experiments conducted in the storage house in which Porto Rico and Big Stem Jersey varieties were used, Yellow Jersey not being available at the time.

TABLE 6.—*The effects of cutting a fresh slice from halved roots that have been exposed to high humidities*

First temperature and humidity readings			Second temperature and humidity readings			Treatment of roots	Number of roots used	Number of roots infected	Percentage infected
Temperature, °C.	Depression of wet bulb, °C.	Relative humidity	Temperature, °C.	Depression of wet bulb, °C.	Relative humidity				
a 19.6	0.23	97	29	2.6	82	Freshly cut...	40	37	93
a 19.6	.23	97	29	2.6	82	Old cut.....	39	2	5
a 22.2	1.4	87	29	2.6	82	Old cut.....	30	16	53
b 28	.53	96	29	2.6	82	Freshly cut...	74	62	84
b 28	.53	96	29	2.6	82	Old cut.....	67	10	15

* These lines of figures are concerned with Porto Rico variety of potatoes.

* These lines of figures are concerned with Big Stem Jersey variety.

Two hundred and twenty halved roots which had been held at relative humidities of 96 and 97 per cent for a period were divided into two lots. A fresh slice, about 2 mm. thick, parallel to the cut surface, was removed from the one lot of 114 halves. The remaining 106 halves were left uncut as controls. Both lots were then placed at a relative humidity of 82 per cent. After 6 and 12 days 99 out of the 114 freshly cut potatoes (or 87 per cent) were infected, while only 12 out of the 106 (or 11 per cent) of the control potatoes became infected. Likewise 30 halved roots that had been held at a relative humidity of 87 per cent were also placed at a relative humidity of 82 per cent. Sixteen of them (or 53 per cent) became infected after 6 days. This latter percentage is high in contrast to that obtained from potatoes held first at relative humidities of 96 and 97 per cent and then placed at a relative humidity of 82 per cent, indicating that there was not as much resistance developed at a relative humidity of 87 per cent as at 96 and 97 per cent.

These results indicate that the resistance shown by sweet potatoes at high humidities is located in the surface layers of the wounded areas, because when this layer is removed the roots become infected readily when subjected to humidities that are favorable for infection. If this layer is not removed very few infections occur, even though the potatoes are placed at humidities which are favorable to infection.

Weimer and Harter (7) found that cork formation, in connection with wounded surfaces of sweet potatoes, took place more readily at high humidities than at low. Hauman-Merck (2) claims that there is some connection between cork formation and infection of sweet potatoes by *Mucor stolonifer* Ehrh. (*Rhizopus nigricans*). It is not unlikely that cork formation interferes with infection at the higher humidities, and that lack of cork formation at the lower humidities permits infection. To be certain that this relation holds it will be necessary to connect these factors more directly than has been done.

EFFECTS OF HUMIDITY ON UNWOUNDED ROOTS

Experiments were conducted during three seasons to determine whether it was possible to keep unwounded sweet potatoes at high humidities and at various temperatures throughout the season. The results are recorded in Table 7. Only 6 out of 350 potatoes held at various temperatures and at humidities ranging between 91 and 97 for 56 days during the autumn of 1921 became infected with *Rhizopus*. Of course, relative humidities are not comparable at different temperatures, but in any case the relative humidities were high. Table 7 gives the depression of wet bulb and humidity at each temperature so that the results can be interpreted directly.

TABLE 7.—*The relation between the exposure of Yellow Jersey sweet-potato roots to high humidities for long period and infection by Rhizopus*

Date and season	Temperature °C.	Depression of wet bulb °C.	Relative humidity	Duration of experiment in days	Number of roots used	Number of roots infected with <i>Rhizopus</i>	Percentage of roots infected with <i>Rhizopus</i>
Oct. 18, 1921-Dec. 13, 1921-----	28.0	1.1	92	56	50	0	-----
	25.5	.7	94	56	50	1	-----
	24.0	.8	93	56	50	0	-----
	20.5	.8	93	56	50	0	-----
	18.5	.6	95	56	50	0	-----
	14.0	.9	91	56	50	0	-----
	11.0	.3	97	56	50	5	-----
Total-----					350	6	1.7
Jan. 1, 1922-May 10, 1922-----	26	1.2	90	114	20	1	-----
	25	.9	93	114	20	1	-----
	24.5	.8	93	114	20	3	-----
	20.5	.5	95	114	20	1	-----
	18.0	.5	95	114	20	5	-----
	12.0	.3	97	114	20	4	-----
	11.5	.5	94	114	20	1	-----
Total-----					140	16	12
Nov. 13, 1922-Dec. 18, 1922-----	28	1.0	93	32	50	0	-----
	25	1.2	90	32	50	0	-----
	23	1.0	91	32	50	0	-----
	20	.2	98	32	50	0	-----
	18	.8	92	32	47	0	-----
	13	1.1	89	32	50	0	-----
	11.5	1.1	88	32	50	0	-----
Total-----					347	0	0
Nov. 13, 1922-Mar. 17, 1923-----	28	1.2	90	124	50	0	-----
	25	1.0	92	124	50	0	-----
	23	1.0	92	124	50	0	-----
	20	.2	98	124	50	0	-----
	18	.8	92	124	47	0	-----
	13	1.1	89	124	50	0	-----
	11.5	1.1	88	124	50	0	-----
Total-----					397	1	.3
Oct. 26, 1923-Dec. 19, 1923-----	27	1.2	90	54	59	0	-----
	25	.7	94	54	74	0	-----
	23	.8	93	54	70	1	-----
	20	.7	94	54	74	0	-----
	18	.5	95	54	68	0	-----
	14	.5	94	54	79	0	-----
	12	.6	94	54	76	0	-----
Total-----	9.6	.2	97	54	77	0	-----
Total-----					577	1	.2
Oct. 26, 1923-Mar. 24, 1924-----	28	.5	97	149	50	8	-----
	25	.7	94	149	74	3	-----
	23	.8	93	149	70	2	-----
	20	.7	93	149	74	0	-----
	18	.5	95	149	67	0	-----
	14	.5	95	149	77	3	-----
	12	.6	94	149	73	0	-----
Total-----	9.6	.2	97	149	77	2	-----
T-----					*571	18	3.2

* The discrepancy between this figure and 577, the number of potatoes used during the fall, is explained by the fact that 5 potatoes were removed for other purposes.

A new lot of potatoes was used the latter part of the same season, and in this case 16 out of 140 roots (or 12 per cent) became infected. Some of the chambers in which the potatoes were stored were opened during the storage period and the temperature allowed to drop to that of the surrounding air. The humidity also dropped considerably during this period. Mice entered through the open doors in some of the chambers and gnawed some of the roots. Eight of the 16 infections took place at the point of gnawing. This fact accounts for at least 50 per cent of the infections. It is entirely possible that more of these infections may have been due to wounding caused by mice that was not macroscopically evident.

During the seasons of 1922 and 1923, 1 out of 397 roots held at constant temperatures ranging between 10° and 28° C. and at relative humidities varying from 88 to 98, became infected after 124 days.

During the season of 1923 and 1924, 19 out of 572 potatoes (or 3.2 per cent) became infected after 150 days. The temperature ranged from 9.6° to 28° C., and the relative humidities from 90 to 97 per cent.

During the three seasons, 42 out of 1,464 (or 3 per cent) became infected with *Rhizopus*. At least 8 of these became infected because of the gnawings of mice. It is believed that in most of the other cases of infection it was the wounding which was the chief predisposing condition for infection by *Rhizopus*, rather than humidity or temperature. The potatoes were handled two or three times during a season, and it is almost impossible to handle sweet potatoes without some wounding. There is nothing in the data in Table 7 to suggest that there is any relation between infection and either temperature or moisture when there is no wounding. There was not a large variation in the humidity, but the infection that did occur bore no relation to this variation. In any case, unwounded sweet potatoes can be kept at high humidities for long periods without becoming infected with *Rhizopus*.

DISCUSSION AND CONCLUSIONS

The optimum relative humidities for infection of sweet potatoes by *Rhizopus* at a temperature of 23° C. range between 75 and 83 per cent. The percentage of infection decreases rapidly as the humidity is raised above 83 per cent or lowered below 75. The decrease is more consistent above 83 per cent than below 75. This consistency may have some relation to the number of times the higher humidities were attained in these experiments in contrast with the lower humidities. Whether there is some disturbing factor at the lower humidities that may account for this irregularity in results is not known.

The consistency of the results, whether considered in connection with the individual experiment or the experiments as a whole, is striking, when it is understood that the percentage of infection is used as the measure of the effects of humidity.

The relation of humidity to infection is similar in the case of the Yellow Jersey and Porto Rico varieties. Big Stem Jersey responded in a similar fashion to the humidities to which it was exposed.

The relation between humidity and infection of sweet potatoes by *Rhizopus* would seem to be governed by reactions on the part of the host and the pathogenes. Why is it that sweet potatoes become infected with *Rhizopus* less readily at humidities above 90 per cent

than at humidities of 75 to 85? Is it because the higher humidities are less favorable to the pathogenes? This is believed not to be the case, for if it were, why do potatoes that have been held at the lower humidities become infected when removed to humidities of 90 and above? It is believed, rather, that some change occurs in the host that renders it immune, because potatoes that have been held at high humidities become infected but rarely when subjected to lower humidities.

In what does this resistance consist? We have a hint from Weimer and Harter's work, "Wound-Cork Formation in the Sweet Potato" (7). They found that cork formation in connection with wounded surfaces occurs more readily at high humidities than at low. The concomitant relation of infection and cork formation to humidity would seem to have some significance, particularly since the resistance exhibited by roots at high humidities is located in the wounded surface. However, further work is required to determine more definitely the relation of cork formation to infection.

Why is it that infection does not take place as readily at relative humidities below 74 per cent as it does at humidities between 75 and 83? This question can not be definitely answered at present. It is believed that it is because moisture becomes a limiting factor to the pathogenes; i. e., these low humidities do not furnish sufficient moisture for the germination of the spores and the penetration of the germ tubes. Of course it is possible that the host may resist infection at these humidities, but it seems unlikely, because these same roots become infected when they are placed at high humidities. At any rate, if the absence of infection is due to the condition of the host, other than the lack of sufficient moisture for the pathogenes, this condition is transitory.

These results may explain the inability of Harter, Weimer, and Adams (1) to obtain infection of sweet potatoes by *Rhizopus* in moist chambers. The humidity in the moist chambers was too high to permit of infection. By use of the "well" method of inoculation they were able to obtain infection in moist chambers. The success of the "well" method of inoculation, may possibly be explained on the basis that the process of infection was thus hastened because of the amount of inoculum used, thereby preventing the development of the condition which prevents infection at high humidities. It is possible by other means (4) to prevent the development of the resistance at high humidities sufficiently to permit of infection. If the roots are severely wounded by striking them over the edge of a wire basket they become infected readily at high humidities. This fact may possibly be explained by Weimer and Harter's work on cork formation in wounded surfaces. They found (7) that when roots were wounded by striking them over the edge of a wire basket, cork formation did not take place at the bottom of the wounds. Such interruption of this process would account for infection in deep and severe wounds at high humidities, even though cork formation normally occurs in an atmosphere of high moisture content.

The success in keeping unwounded sweet potatoes at high humidities throughout the season without their becoming infected can be explained on the basis that *Rhizopus* rarely infects sweet potatoes except through fresh wounds.

It is possible that if freshly dug potatoes—which of course are freshly wounded—were placed at low humidities, they might become infected if they were exposed to high humidities later. Experiments are now under way to determine whether this is true.

Weimer and Harter's work (7) suggests that excessive drying of freshly dug sweet potatoes renders them more susceptible to the attack by *Rhizopus*. How often sweet potatoes are excessively dried during the process of curing is not known.

SUMMARY

The data recorded in this paper, and those obtained from other experiments, show that infection of sweet potatoes by *Rhizopus* occurs almost exclusively through fresh wounds.

The optimum relative humidities at a temperature of 23° C. for the infection of halved roots of sweet potatoes by *Rhizopus* range from 75 to 84 per cent.

The percentage of infection decreases rapidly as the humidity is raised above or lowered below the optimum humidities.

This decrease is more consistent above the optimum than below it; i. e., there is less variation in the number of infections above the optimum than below it.

Very few infections of halved roots occurred at relative humidities of 93 to 99 per cent at a temperature of 23° C.

Very few halved roots which had been subjected to relative humidities of 89 to 97 per cent (temperature 23° C.) for a period of 4 to 12 days became infected when they were retained at these humidities for another period or were placed at humidities of 48 to 89 per cent.

On the other hand, a high percentage of the halved roots which were subjected to relative humidities of 51 to 73 per cent for a period of 4 to 12 days became infected when they were placed later at relative humidities of 84 to 95 per cent.

Halved roots that had been exposed to relative humidities of 51 to 52 per cent for a period of 9 to 12 days did not become infected when they were subsequently placed at relative humidities of 48 to 73 per cent.

A resistance to infection by *Rhizopus* developed in the halved roots that had been held at relative humidities of 89 to 97 per cent.

This resistance is located in the surface layers of the wounded areas and has a possible relation to cork formation.

The Yellow Jersey, Big Stem Jersey, and the Porto Rico varieties of sweet potato responded in a similar fashion (within the range of humidities used in each case) to the action of air moisture.

The Yellow Jersey variety was kept for periods of 56 to 150 days during three seasons with only a few infections with *Rhizopus* occurring.

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THE USE OF SODIUM NITRITE IN THE CURING OF MEAT¹

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INTRODUCTION

The art of preserving meats by curing in common salt, with or without smoking, is one which has been practiced from remote antiquity. Directions for curing meats with salt appear in ancient writings, and the art is still practiced among primitive peoples. Yet, notwithstanding the antiquity of the art and its economic importance, adequate and comprehensive scientific study with publication of results has never been given to it. Even at the present time the scientific publications on the subject consist largely of recipes and formulas or the results of desultory and isolated experiments.

The use of nitrates also dates from ancient times, and has been practiced so long that its origin is unknown (2, p. 613)². Only within recent years, however, has the function of nitrates been correctly understood. The investigations of Haldane (1) and Hoagland (3, 4) demonstrated that this function is the fixation of color which takes place through the reduction of nitrate to nitrite, and the combination of the latter with the hemoglobin of the meat. McBryde (7) showed that potassium nitrate has no more preservative power than common salt when tested against the organisms concerned in the spoilage of hams. Present scientific knowledge, therefore, indicates that nitrates are useful only as color fixatives and that their aid in preservation is not of importance.

The nitrates, as such, are without value as color fixatives and become active only when reduced to nitrites. In practice this is proved by the fact that if the formation of nitrites is prevented or interfered with in any way the color fixation is likewise prevented or interfered with. Unsatisfactory or insufficient formation of nitrite invariably results in unsatisfactory, inadequate, or irregular fixation of color. So far as color fixation is concerned, the progress of curing in a vat of meat in pickle may be followed by determining the nitrite content of the pickle.

The formation of nitrite was ascribed by Haldane (1) to bacterial action. This opinion is evidently correct, as there are several circumstances connected with practical meat curing which are best explained on the assumption that the nitrate is reduced by bacterial action. Among these are the variability of the cure, often observed in a new establishment, the advantageous results obtainable by adjustment and control of the hydrogen-ion concentration of the pickle, the benefits derived from "seeding," and the occasional failure of nitrite formation caused by development of acid-forming organisms in the pickle.

¹ Received for publication Apr. 20, 1926; issued September, 1926.

² Reference is made by number (italic) to "Literature cited," p. 551.

The source of the nitrate-reducing organisms has never been definitely ascertained, but in all probability it is multiple. Nitrate-reducing organisms are so widely distributed in nature that it would be difficult to find an environment in which they are not present. From general knowledge of the occurrence of such organisms it is to be presumed that the water used in making up the pickling solutions, the tables, trucks, and other equipment employed in handling the meats, the curing vats and boxes, and the meats themselves, are sources of nitrate-reducing organisms. It has been observed that organisms capable of effecting the conversion of nitrate to nitrite in the presence of such concentrations of salt as are commonly used in meat curing can be readily isolated from the fresh pickling solutions. Considerable variability in the vigor of growth and the rapidity and completeness of the conversion effected has been observed among different isolations. Other observations indicate that the curing vats are constant and reliable sources of the active nitrate-reducing organisms. Although it is customary to steam out the vats after use, it is known that this steaming does not result in sterilization, and a vat once well inoculated with a vigorous and effective nitrate-reducing organism would doubtless harbor the organism for an indefinite and extended period. The uniformity of curing action which is often attained and maintained for long periods is explainable on this basis.

The curing action proceeds, as a rule, with remarkable uniformity and regularity. An example of the typical course of nitrite formation is given in Table 1.

TABLE 1.—*Changes in nitrite content of nitrate pickle*

Day	NaNO ₂ (parts per million)	Day	NaNO ₂ (parts per million)
1st.....	3.6	41st.....	421.0
5th.....	10.0	48th.....	540.0
15th.....	33.0	55th.....	700.0
26th.....	138.0	62d.....	820.0
30th.....	189.0	65th.....	887.0

In contrast to the typical and satisfactory course of nitrite formation cited above, various degrees of unsatisfactory formation, ranging from delayed formation necessitating a brief prolongation of the curing period to nearly total failure of nitrite formation, have been observed.

Any deviation from the normal process is, of course, a source of loss, inconvenience, and expense. Means of eliminating the irregularities of nitrate formation and obtaining uniformity of curing have naturally been much sought. The means employed and the results obtained have not been published, but have been held as trade secrets by the various establishments using them. Consideration of the problem indicates that it may be solved either by the cultivation of a suitable nitrate-reducing organism in the pickle or by the direct addition of the required nitrite as such instead of depending on the formation by bacterial action. The first method, while unquestionably feasible, has the disadvantage of requiring expensive supervision, and is not applicable in the absence of a scientific laboratory. This method is therefore open only to the larger establishments. No ad-

vantage over the direct use of nitrite either from the standpoint of operations or of public health is apparent. The direct use of nitrite is therefore suggested as the method of choice. The experiments described herein were undertaken in order to determine the practicability of using nitrite directly and the advantages and disadvantages connected with such use. On the part of the bureau the possibility of harm to human health was of course given primary consideration.

EXPERIMENTS IN DIRECT USE OF NITRITE

The first experiment involving the direct use of nitrite was formally authorized January 19, 1923, as a result of an application by one of the large establishments operating under Federal meat inspection. Before that time other requests for permission to experiment with nitrite had been received but had not been granted. The authorization for the first experiment specified that the whole process was to be conducted under the supervision of bureau inspectors and that after the curing had been completed the meat was to be held subject to laboratory examination and final judgment and would be destroyed if found to contain an excessive quantity of nitrites or if in any way it was unwholesome or unfit for food. This principle was rigidly adhered to throughout the experimental period, no meat being passed for food until its freedom from excessive nitrites had been assured, either by laboratory examination or through definite knowledge from previous examinations, that the amount of nitrite used in the process would not lead to the presence of an excessive quantity of nitrites in the meat.

By "excessive" is meant a quantity of nitrite materially in excess of that which may be expected to be present in similar meats cured by the usual process. The analyses given in Table 2 are typical of the nitrite content of meats cured with nitrate in accordance with the customary practice.

TABLE 2.—*Nitrite content of meats cured by nitrate process*

Kind of meat	NaNO ₂ (parts per million)	Kind of meat	NaNO ₂ (parts per million)
Ham (whole slice, ground)	72.0	Ham	60.0
Do.	40.0	Ham (outside portion of piece)	480.0
Do.	88.0	Ham (inside portion of piece)	192.0
Do.	104.0	Ham (outside portion of piece)	624.0
Corned beef	3.6	Ham (inside portion of piece)	288.0
Do.	2.4	Ham (outside portion of piece)	576.0
Do.	41.0	Ham (inside portion of piece)	480.0
Do.	24.0	Ham (outside portion of piece)	960.0
Do.	12.0	Ham (inside portion of piece)	576.0
Do.	12.0	Bacon (outside portion of piece)	120.0
Salt Pork	7.2	Bacon (inside portion of piece)	104.0
Do.	2.4	Bacon (outside portion of piece)	640.0
Dried beef	7.2	Bacon (inside portion of piece)	624.0
Do.	12.0	Bacon (outside portion of piece)	144.0
Boiled ham	12.0	Bacon (inside portion of piece)	72.0
Do.	17.0	Bacon (outside portion of piece)	120.0
Do.	24.0	Bacon (inside portion of piece)	96.0
Salt pork	2.4	Bacon (outside portion of piece)	96.0
Do.	208.0	Bacon (inside portion of piece)	56.0
Do.	14.4	Bacon (outside portion of piece)	336.0
Do.	240.0	Bacon (inside portion of piece)	288.0
Smoked shoulder	180.0	Dry-salt butt	1.5
Salt pork	2.4	Do.	2.9
Ham	60.0	Dry-salt fatback	1.9
Corned beef	60.0	Do.	1.5
Ham	48.0	Boiled ham	16.0
Do.	36.0	Smoked ham	568.0

Under the terms of the initial authorization, 92 pieces of meat weighing in all 1,142 pounds were cured in pickle containing approximately 0.2 per cent of sodium nitrite, and 94 pieces of bacon, weighing 1,270 pounds, were cured in boxes with a dry mixture containing sodium nitrite. These experiments were paralleled by like quantities of meat cured in pickle and with a dry mixture containing the usual proportions of sodium nitrate. Analyses were made of the finished meats, as well as of the curing materials used, and of the pickles at different ages. The finished meats were also tested by cooking and serving to a considerable number of persons, of whom some knew that an unusual process of curing had been used, and some did not. The judges were all department employees, and included trained scientists, experienced meat inspectors, and others familiar with commercially cured meats.

Close attention was given to the quality and flavor of the finished meats as well as to nitrite content throughout the experiments, since it is obvious that any change in the customary curing process which resulted in impairing the quality of the product would not be in the public interest. The general plan outlined above was followed throughout the experimental period.

The changes in the nitrite content of the pickle in two vats of hams in nitrite cure are shown (Table 3) for comparison with the corresponding changes in a typical nitrate cure (Table 1).

TABLE 3.—Changes in nitrite content of nitrite pickle

Day	NaNO ₂ (parts per million) in—		Day	NaNO ₂ (parts per million) in—	
	Vat A	Vat B		Vat A	Vat B
1st.....	1,820	1,820	48th.....	864	824
5th.....	1,365	1,365	55th.....	850	790
15th.....	1,259	1,213	62d.....	810	720
26th.....	1,100	1,040	65th.....	730	720
30th.....	1,058	1,024	68th.....		
41st.....	8.6	830			

An examination of five of the hams cured with sodium nitrite showed an average nitrite content ranging from 42 to 150 parts per million. The nitrite content was found to be greatest in that part of the ham nearest to the flesh side and least in the center next to the bone. The maximum nitrite content of any part of any nitrite-cured ham was 200 parts per million. The hams cured with nitrate in the parallel experiment showed a maximum nitrite content of 45 parts per million.

From the standpoint of flavor and quality the results were satisfactory. All judges agreed that the nitrite-cured meat was fully equal in quality and flavor to that cured by the customary process. No deficiency in quality or flavor was noted by any of them, and none of them were able to distinguish the nitrite-cured meat from that cured by the customary process.

In addition to the curing of hams in sweet pickle, the initial experiment covered the curing of bacon, both in sweet pickle and by the box process. The results with both types of bacon were

also successful. The initial experiment therefore demonstrated that hams and bacon could be successfully cured with sodium nitrite, and that nitrite curing need not involve the presence of as large quantities of nitrite in the product as sometimes are found in nitrated-cured meats.

Following the successful outcome of the initial experiment, further experiments were authorized, first at the original establishment and subsequently at others. In all, experiments were conducted at 17 establishments in 13 cities. These establishments ranged in size from two of the largest in operation to those of rather small size, and included some at larger centers of consumption as well as several in the producing sections. The products cured included pork shoulders, shoulder butts, pork loins, pork trimmings, pork tongues, beef tongues, lamb tongues, corned beef, and dried beef, as well as hams and bacon. Representatives of the Institute of American Meat Packers participated in the experiments at certain establishments. Their results have been published elsewhere (5, 6). The duration of the experiments ranged from two years and eight months at the first establishment to a few weeks at the last establishment permitted to conduct experiments. The quantity of meat cured with sodium nitrite was large, as all establishments were permitted to make their experiments on a commercial scale. The results published here were therefore derived from full-scale operations and are directly applicable to commercial meat curing.

Supervision of the experiments followed the plan outlined in the first experiment. No control was exercised over the curing formulas except that a rather liberal limit was set to the quantity of nitrite which might be used and the establishment was not permitted to exceed that limit. The first experiment having shown it to be easily attainable, 200 parts per million was fixed as a tentative limit for the nitrite content of the finished meats.

Results of large-scale operations confirmed those of the first experiment. It was demonstrated at each establishment which conducted experiments that sodium nitrite could be successfully substituted for the sodium or potassium nitrate customarily used, and that the direct use of nitrites did not involve any increase in nitrite in the finished meat. Results for each establishment will not be given, since this would involve a needless repetition.

All operations were conducted under the direct supervision of Federal inspectors. Packing-house officers and employees, however, arranged for and conducted the actual handling of the meat.³

The results obtained with hams in sweet-pickle cure at two establishments at which nitrite-curing experiments were conducted on a considerable scale are given in Table 4.

³ The writers acknowledge the excellent cooperation of the many Federal inspectors and packing-house employees who participated in this work and who furnished the data on which the results here reported are based.

TABLE 4.—*Sodium nitrite in corresponding samples of pickle and of meat*

Establishment A. NaNO ₂ (parts per million)		Establishment B. NaNO ₂ (parts per million)	
Pickle	Meat	Pickle	Meat
500.....	20	450.....	60
450.....	20	600.....	30
630.....	30	500.....	50
650.....	35	650.....	70
625.....	50	600.....	30
550.....	40	500.....	70
600.....	35	650.....	70
600.....	50	600.....	70
550.....	40	650.....	60
600.....	30	550.....	60
550.....	40	450.....	40
450.....	30	650.....	100
400.....	20	550.....	60
400.....	80	600.....	80
375.....	30	400.....	70
450.....	80	650.....	70
450.....	50	550.....	80
400.....	90	450.....	90
550.....	95	600.....	85
600.....	90	550.....	70
450.....	100	600.....	90
550.....	60	550.....	90
550.....	65	600.....	85

The first column shows the nitrite strength of the pickle at the conclusion of the curing period. The second shows the nitrite content of the meat cured in the pickle represented by the corresponding sample. The nitrite content of the pickle at the conclusion of the curing period ranges from 375 to 650 parts per million. This corresponded to a nitrite content of from 20 to 100 parts per million in the smoked hams. These samples were collected at approximately monthly intervals during a period of 18 months and show results of large-scale operations.

Comparable results were obtained on the other products cured with sodium nitrite. In no case and in no establishment did the nitrite content of the finished product exceed, or regularly approach, the tentative limit of 200 parts per million, after the employees of the establishment had become familiar with the use of nitrite.

Some differences were noted in the application of nitrite curing to individual products, although the underlying principles, of course, are applicable to all products. Curing in all cases and in all products consists in the absorption of sufficient salt to preserve the meat, the fixation of color, and the development of that distinctive flavor and texture which distinguishes cured from fresh meats. If any one of these three factors fails the results are unsatisfactory. Differences in curing methods for different products are determined by the size, shape, and character of the different cuts and products and to a less degree by the salt content and flavor desired in the finished product.

The irreducible minimum of time in all curing is that period required for the meat to take up the required quantity of salt. In curing with nitrates the absorption of salt runs ahead of the fixation of color, since salt penetration begins as soon as the meat is placed in cure, while color fixation can not begin until nitrite is formed. In nitrate curing, therefore, the meats must be held in cure for a more or less extended period after they have taken up the necessary salt. By the use of nitrite this additional period of waiting for color fixation

is avoided, as color fixation, like salt penetration, begins at once. For this reason the use of nitrite makes possible a material shortening of the curing period. In actual practice, with one exception, a shortening of the curing period was realized at all of the establishments in which experiments were conducted. The exception was an establishment in a large consuming center, which had been using an exceptionally rapid curing process. Although no reduction of the curing period was accomplished in this instance, the quality of the product was materially improved. In one establishment the shortening amounted to 60 per cent of the former curing period; in the other establishments the shortening amounted to from 10 to 40 per cent of the former curing period.

The flavor and quality of the meat cured with nitrite was in all cases at least equal to that cured with nitrate by the process regularly used by the establishment. In one case no improvement could be detected, the nitrite-cured meat being identical with that cured by the establishment in the regular way. In all other cases an improvement in quality was discernible after the nitrite-curing process had been established on a working basis.

The quantity of sodium nitrite required for successful fixation of color varies according to the process employed. One-fourth of an ounce, or less, of sodium nitrite appears to be sufficient to fix the color of 100 pounds of meat. In case of chopped meats, such as trimmings, where all the curing materials added remain in the meat and none is lost in the form of unused pickle or residual liquid, this proportion has been found to be sufficient. In other products the use of somewhat more nitrite is required. In any case 1 ounce of sodium nitrite appears to be sufficient for 100 pounds of meat. The minimum quantity used in any experiment in the curing of hams in sweet pickle corresponded to $6\frac{2}{3}$ ounces of sodium nitrite to 100 gallons of pickle, or approximately $\frac{1}{2}$ ounce to 100 pounds of meat. This proportion was insufficient for satisfactory color fixation, as also was a pickle containing $13\frac{1}{2}$ ounces in 100 gallons, or approximately $\frac{3}{4}$ ounce to 100 pounds of meat. On the other hand, a pickle containing $\frac{1}{2}$ pound of sodium nitrite in 100 gallons, or a little less than $\frac{1}{2}$ ounce to 100 pounds of meat, was used with success at another establishment. In the latter case, however, the hams were pumped with a stronger pickle, so that the quantity of nitrite actually added to the meat is not accurately known and was more than that contained in the pickle. From the information available it appears that the nitrite strength of either curing or pumping pickles should not be less than $\frac{1}{2}$ pound or more than 2 pounds to 100 gallons. This applies to all products cured in pickle, either plain or sweet; that is, with or without sugar.

At one establishment hams cured in a nitrite pickle were also pumped with a pickle containing a large quantity of nitrate. The pumping pickle was also of high salt strength. The nitrate injected with the pump pickle was found to remain in the ham without material change throughout the curing process, so that the hams at the completion of the cure contained only such nitrites as were derived from the curing pickle. After cutting and exposure of the cut surfaces to the air, rapid reduction of nitrate took place, so that within a short time the meat contained large quantities of nitrites. At the

same time a decidedly disagreeable flavor was developed. After applying the obvious remedy, namely, replacement of the nitrate-pumping pickle with one containing a suitable proportion of nitrite, further results were satisfactory.

At several other establishments nitrite and nitrate were tried in combination. No advantage was found to result from the combination in any case. If nitrite is used at all, it appears desirable to employ a sufficient amount to fix the color of the meat. If this is done, no advantage from the addition of nitrate is evident.

Several establishments used nitrite in the curing of bacon by the box process. This process consists in packing the bacon in a water-tight box with the curing mixture. Although often referred to as a dry-curing process, it is not that in fact, as the curing mixture within a very short time forms a solution through extraction of fluid from the meat, and the meat is thereafter immersed in the liquid thus formed. The process is therefore "dry" only in the sense that no fluid is added. The proportions of nitrite used ranged from $\frac{1}{4}$ ounce to $1\frac{1}{4}$ ounces to each 100 pounds of meat. All proportions used were successful, the smallest being sufficient for satisfactory color fixation while the greatest did not result in excessive nitrite in the meat. The experiments demonstrated the necessity of thorough mixing of the curing ingredients, as in one instance the meat was damaged by local action of nitrite resulting from imperfect mixing. Each establishment which experimented with this process accomplished a material shortening of the curing period.

In curing pork loins with nitrite one establishment failed to obtain a satisfactory color and attempted to get better results by increasing the proportion of nitrite. The attempt proved unsuccessful, and it was found that the failure was caused by a lack of hemoglobin in the meat rather than of nitrite in the curing mixture. In this instance $\frac{1}{4}$ ounce of sodium nitrite was found to be more than sufficient to secure the maximum possible color in 100 pounds of pork loins. The results of this experiment are of particular interest in showing that a lack of color in the product may be caused by a lack of sufficient hemoglobin rather than by a lack of nitrite, and that when such is the case it is of no avail to increase the proportion of nitrite.

Experiments involving the use of nitrite in smoked and cooked sausage were of particular interest. In the manufacture of sausage of this type it is customary to use a curing process. In this process all or part of the trimmings may be cured before chopping, or the curing agents may be added to the chopped meats and the mixture allowed to stand long enough, under suitable conditions, to attain the effect desired. In either case the use of nitrate is customary and the formation of nitrite and fixation of color are essential parts of the curing process. The curing period being in all cases short, the proportion of nitrate commonly used is liberal. This may result in the presence of considerably more nitrite than is required in the finished product. In Table 5 are given the results of a survey which included all brands of sausage of the Frankfurter style and imitation sausage resembling Frankfurter style prepared at establishments operating under Federal inspection at one meat-inspection station of the bureau.

TABLE 5.—Nitrite content of Frankfurter style sausage and imitations prepared with nitrate

Sample No.	Establishment	Product	NaNO ₂ (parts per million)
5475	A	Imitation Frankfurter.....	Trace.
5476	A	Frankfurter style sausage.....	30
5578	B	Imitation Frankfurter.....	160
5580	B	Frankfurter style sausage.....	160
5581	B	do.....	160
5582	B	do.....	250
5583	B	do.....	625
5642	C	Imitation Frankfurter.....	20
5643	D	Frankfurter style sausage.....	1,400
5644	D	Imitation Frankfurter.....	50
5645	C	Frankfurter style sausage.....	20
5682	E	Imitation Frankfurter.....	140
5683	E	Frankfurter style sausage.....	500
5684	E	do.....	250

Nitrate was used in the preparation of the products represented by all of the samples shown in Table 5. The quantities of nitrate were such as are commonly employed, and no unusual process of curing or manufacture was used. The results, therefore, are typical of what may be expected from the ordinary and usual processes of sausage manufacture. An examination of a large number of samples collected from numerous establishments in different cities has shown that the variations in nitrite content indicated in Table 5 are typical and not exceptional.

The experiments involving the use of nitrite in sausage showed that the curing period can be materially shortened. The nitrite being added in the form of the pure salt, the quantity can be accurately controlled and the variation in the nitrite content of the product prevented. It was found that one-fourth ounce or less of sodium nitrite is sufficient to fix the color in 100 pounds of sausage meat. The results shown in Table 6 are typical of the nitrite content of sausage prepared with sodium nitrite.

TABLE 6.—Nitrite content of sausage prepared with nitrite

Sample No.	Establishment	Product	NaNO ₂ (parts per million)
1211	E	Frankfurter style sausage.....	40
4527	F	Bologna style sausage.....	60
2886	G	Frankfurter style sausage.....	150
2898	G	Luncheon meat.....	80
3627	G	Minced luncheon.....	50
3628	G	Frankfurter style sausage.....	50
3646	G	do.....	60

It is evident from Table 6 that the nitrite content of sausage can be controlled within very much narrower limits when nitrite is used instead of nitrates.

EFFECT OF THE USE OF NITRITE ON THE PUBLIC HEALTH

During the whole course of the experiments the question of possible harm or benefit to the health of the public was given primary consideration. The presence of nitrites in cured meats, however, was already sanctioned by the authoritative interpretation of the meat inspection and pure food and drugs acts sanctioning the use of saltpeter (9); as shown previously, meats cured with saltpeter and sodium nitrate regularly contain nitrites. The questions to be decided, then, were whether sodium nitrite could be successfully substituted for saltpeter or sodium nitrate, whether the residual nitrite in the meats cured with sodium nitrite would be greater than that in meats cured in the usual manner, and whether the meats cured with sodium nitrite would in any way be less wholesome than those cured by the usual process. The results have shown that nitrites can be successfully substituted for nitrates. No curing difficulties caused by the use of nitrites were observed. At no establishment was any increase in the proportion of spoilage observed. The residual nitrites found in the nitrite-cured meats were less than are commonly present in nitrate-cured meats. The maximum quantity of nitrite found in nitrite-cured meats in particular was much smaller than the maximum resulting from the use of nitrate. The nitrite-cured meats were also free from the residual nitrate which is commonly present in nitrate-cured meats. No other condition or character was observed in the nitrite-cured meats which could possibly make them any less wholesome than if cured with nitrate. No objection to the substitution of nitrite for nitrate from the standpoint of public health was therefore disclosed by the experiments. On the contrary, the more accurate control of the amount of nitrite and the elimination of the residual or unconverted nitrate are definite advantages attained by the substitution.

SUMMARY AND CONCLUSIONS

Sodium nitrite can be successfully substituted for sodium or potassium nitrates in the curing of meat.

From one-fourth to 1 ounce of sodium nitrite is sufficient to fix the color in 100 pounds of meat, the exact quantity depending on the meat to be cured and the process to be employed.

Meats cured with sodium nitrite need contain no more nitrites than meats cured with nitrates, and are free from the unconverted nitrates regularly present in nitrate-cured meats.

A shortening of the customary curing period may be obtained by the use of nitrite.

Meats cured with sodium nitrite in the proper quantity and in accordance with sound practice are in no way inferior in quality or wholesomeness to meats cured with nitrates.

As a result of the experiments described above, the use of sodium nitrite in meat curing in Federally inspected establishments has been formally authorized by the Department of Agriculture (8), subject to meat-inspection regulations.

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ELECTRODIALYSIS OF THE COLLOIDAL SOIL MATERIAL AND THE EXCHANGEABLE BASES¹

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INTRODUCTION

Recent investigations in the Bureau of Soils have shown that the colloidal material present in any soil contains 10 or more elements in widely varying proportions (19).² Various attempts to fractionate the material into substances of more definite composition have not met with much success. However, dialysis of the material indicated that one colloid held about 1 per cent of its constituents less firmly than the remainder. Long-continued treatment apparently removed all the lime, soda, and sulphur but only a small part of the magnesia, potash, and other elements.

It was thought that further evidence concerning the firmness with which the different constituents are held in the colloid might be obtained by electrodialyzing the material. Electrodialysis is similar to ordinary dialysis, but is much more rapid and effects a separation of the diffusible ions of opposite charge.

The mass of data that has been accumulated on base exchange in soils indicates that the colloidal soil material must contain a fairly definite part of its bases in a reactive condition. It was therefore important to compare the bases that can be removed by electrodialysis with the quantity removable by base exchange with a salt solution.

PREVIOUS WORK

While electrodialysis has been used in other fields of investigation, only a few attempts appear to have been made to apply it to the study of soils. Cameron and Bell (4) studied the results of continuously removing the products of hydrolysis from powdered hornblende, apatite, etc., by the use of the electric current. Gradually decreasing, small amounts of monovalent and divalent bases were thus removed. It is therefore evident that the minerals of a soil would be attacked to a slight extent by this treatment.

König, Hasenbäumer, and Hassler (15) and later König, Hasenbäumer, and Kuppe (16) studied the amount of material removable from different soils by the electric current and found that there was some correspondence between the material removed by electrodialysis and that removed by steam and hydrogen peroxide.

The study of base exchange in soils, which dates back to Way (20), has lately received new impetus through the work of Gedroiz (8) in

¹ Received for publication Apr. 24, 1926; issued September, 1926.

² Reference is made by number (italic) to "Literature cited," p. 566.

Russia, Kappen (17) in Germany, Hissink (13) in Holland, and Kelley and Brown (14) in this country. The following facts established in numerous investigations bear on the subject dealt with in this paper. It has repeatedly been shown that in place of the bases removed from a soil by treating it with a neutral salt solution an approximately equivalent quantity of the cation of the salt is adsorbed. As to the quantity of exchange, it has been shown that while this varies in any single treatment with the concentration and volume of the solution and with the kind of cation, approximately the same end point is reached when different salts and concentrations are used, provided the soil is treated a sufficient number of times. This indicates that a given soil contains a fairly definite quantity of bases present in a particularly reactive condition.

These facts were established for the whole soil, but it was generally believed that the reactive bases were present in the colloidal material and this is now known to be so. A recent study (1) shows that the adsorbing power of a soil for various substances is practically all due to the colloidal fraction, and recent work of Kelley and Brown (14) indicates that this is also true in the case of exchange adsorption with inorganic salts.

Since practically all the investigations of base exchange were conducted on whole soils in which neither the quantity nor composition of the colloid was known, no data are available to show what part of the total bases in the colloid are exchangeable.

METHODS

For the following work colloidal fractions of the Sharkey clay soil and the Norfolk fine sandy loam,³ prepared by the supercentrifuge method as explained in a previous publication (10, p. 16), were selected. These two colloids in chemical composition and colloidal properties are representative of the wide differences that have been encountered in the examination of a large number of soil colloids

(19). The Sharkey colloid is gray in color, has a high $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio, a high content of monovalent and divalent bases, a high adsorptive capacity for ammonia gas and basic dyes, and is strongly electronegative as measured by the amount of methylene blue required to render a given quantity of the clay isoelectric. The Norfolk colloid is yellow, has a low $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio, a low content of monovalent and divalent bases, a low adsorptive capacity, and is weakly electronegative.

Table 1 gives the composition of these two soil colloids essentially as determined by the fusion method of the Association of Official Agricultural Chemists (3). Carbonates were absent in both samples, and easily soluble salts, which were low in the original soils, were further reduced by the large quantities of distilled water used in the separation of the colloidal material.

³ The sample of Sharkey clay soil was taken to a depth of 4 inches, near Valley Park, Issaquena County, Miss. The sample of Norfolk fine sandy loam was taken from 12 to 36 inches, one-fourth mile south of Scottsville, Wayne County, N. C.

TABLE 1.—Analysis of the Sharkey clay and Norfolk fine sandy loam soil colloids

Kind of colloid	SiO ₂	TiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MnO	CaO	MgO	K ₂ O
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Sharkey.....	52.05	0.51	22.52	8.12	0.035	1.36	2.52	1.88
Norfolk.....	39.25	.82	33.61	11.24	.005	.30	.44	.49

Kind of colloid	Na ₂ O	P ₂ O ₅	SO ₃	Ignition	Total	Organic	Mols. SiO ₂ Mols. Al ₂ O ₃ & Fe ₂ O ₃
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Sharkey.....	0.21	0.64	0.20	9.99	100.05	3.92	3.18
Norfolk.....	.25	.23	.05	14.04	100.73	1.90	1.63

The exchangeable bases in the colloid were determined by leaching with normal NH_4Cl , essentially as proposed by Hissink (13) for soils, and also by treating with 0.05 normal HCl , as recently proposed by Gedroiz (9). Briefly, the procedure for extraction with NH_4Cl was as follows: A 10 gm. sample of the colloid was shaken up with an ammonia chloride solution and then leached with 1 liter of hot normal NH_4Cl . The bases were determined in the filtrate. The procedure for extraction with 0.05 normal HCl was similar, except that 5 gm. of colloid were used and 300 c. c. of acid.

The apparatus used for electro-dialysis was a three-chambered cell. The central chamber containing the soil material was separated from the outer electrode chambers by means of parchment paper. The cell was constructed by bolting together three U-shaped pieces of soft plate rubber (B_1 , C , B_2) and two sheets of parchment paper between two hard rubber plates (A_1 , A_2) as shown in Figure 1. The electrodes (E_1 , E_2) were of platinum wire gauze and were large enough to extend to the sides and bottom of the chambers. A lattice of glass rods, not shown in the figure, was placed in each of the electrode chambers to support the parchment and prevent bulging when the central compartment was filled with the soil material. The electrode chambers were provided with outlets (O_1 , O_2)⁴ to facilitate the withdrawal of the electro-dialysates.

This form of cell was found to be very satisfactory for the electro-dialysis of soils. The large area of the electrodes and the thinness of the central soil chamber reduced the resistance so that when a

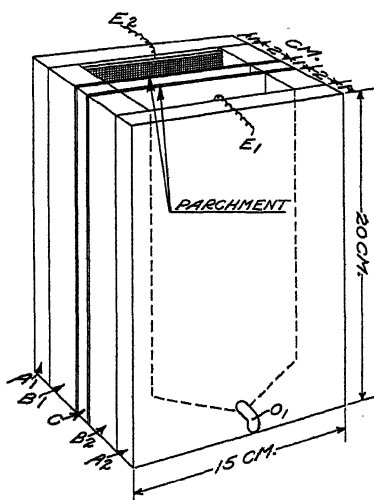


FIG. 1.—Apparatus used for the electro-dialysis of soils and soil colloids

⁴ Outlet O_2 is on the side opposite to O_1 but is not shown in the figure.

220-volt direct current was applied to the cell with a 50 to 25 watt lamp in series the temperature did not rise above 50° C. With a central chamber of the dimensions given in Figure 1, approximately 100 gm. of soil or 60 gm. of the colloid could be electrodialed at one time.

The parchment paper always contains impurities, especially sulphates. These were removed by filling all the chambers with water and allowing the current to flow for some time with a few changes of water.

The removal of anions is somewhat retarded by the electronegative charge of the soil and parchment membrane. The water at the interfaces being correspondingly electropositive, there is an electro-osmotic movement of the liquid toward the cathode which impedes the migration of the anions. The use of an electropositive chrome-gelatin membrane on the anode side has been recommended by Freundlich (7) to nullify this one-sided electrosmose. There does not seem to be any necessity for using such a membrane when soil materials, which contain a considerable excess of diffusible cations over anions, are subjected to prolonged treatment. It was found that the same proportions of bases and acids were removed from a soil whether the anode membrane was positive or negative. Furthermore, on electrodialed a dilute solution of K_2SO_4 it was found that the retardation of the anions is only temporary and does not prevent a quantitative separation. The alkalinity of the cathode solution was considerably higher than the acidity of the anode solution at the end of the first half hour; but after another half hour, when the electrodiagnosis was completed, a quantitative relationship obtained.

PRELIMINARY EXPERIMENTS ON ELECTRODIALYSIS

Before making quantitative determinations on the colloidal soil material, preliminary tests were conducted with two untreated soils in order to gain an idea of the approximate time required for the electrodiagnosis to reach an end point. The two soils tested were the Sharkey clay, which is high in monovalent and divalent bases, and the Manor loam, which is comparatively poor in these bases.

The rate at which material was removed was determined by withdrawing the electrodiagnosisates from the cathode and anode chambers hourly and titrating the bases and acids with phenolphthalein as an indicator. A 78.35 gm. sample of the Sharkey soil and a 98.85 gm. sample of the Manor soil were electrodialed. The specific conductivity in reciprocal ohms of the Sharkey soil in the pasty condition at 25° C. was 1287×10^{-6} before and 286×10^{-6} after electrodiagnosis, while the conductivity of the Manor soil under the same conditions was only 152×10^{-6} before and 24×10^{-6} after the treatment.

Table 2 shows the milliequivalents of titrable bases and acid removed hourly from the two soils.

TABLE 2.—Bases and acids removed hourly by electrodialysis of two soils

Time of electrodialysis	Sharkey clay soil			Manor loam soil		
	Base	Acid	Appearance of cathode dialysate	Base	Acid	Appearance of cathode dialysate
Hours	Milli-equivalents	Milli-equivalents		Milli-equivalents	Milli-equivalents	
1.....	2.655	0.890	Clear.....	1.810	0.152	Clear.
2.....	2.340	.255	do.....	1.180	.062	Light-colored sediment.
3.....	2.282	.205	do.....	.410	.050	Do.
4.....	2.260	.175	do.....	.110	.042	Reddish sediment.
5.....	2.710	.320	do.....	.043	.040	
6.....	2.060	.090	do.....			
7.....	2.015	.090	do.....			
8.....	1.995	.090	do.....			
9.....	1.900	.085	do.....			
10.....	1.810	.085	do.....			
11.....	1.615	.085	do.....			
12.....	1.830	.250	Light-colored sediment.			
13.....	.815	.075	do.....			
14.....	.550	.070	do.....			
15.....	.420	.065	do.....			
16.....	.360	.070	do.....			
17.....	.300	.065	Reddish sediment.			
18.....	.260	.070	do.....			
19.....	.410	.190	do.....			
20.....	.140	.060	do.....			
21.....	.120	.060	do.....			
22.....	.090	.060	do.....			
Total...	28.937	3.375		3.553	.346	

* These relatively high values are the result of standing overnight with no current passing.

It will be noticed that the quantity of titrable, or soluble, base removed hourly from the Sharkey clay soil remains high during the first 12 hours and then falls sharply, but is still determinable at the twenty-second hour. In the case of the Manor loam, a sharp decrease is evident at the end of the second hour. Coincident with the rapid falling off in the quantity of soluble bases, the cathode solution becomes turbid. This turbidity is at first light colored and consists chiefly of $Mg(OH)_2$ and $Al(OH)_3$. Later it becomes brownish due to $Fe(OH)_3$. The appearance of the insoluble bases is evidently governed by the hydrogen-ion concentration in the central chamber containing the soil. The electrodialysis of the Sharkey clay was in one case interrupted at the point where a sediment began to appear, and the P_H , as far as could be determined colorimetrically in the rather turbid soil extract, was found to be 5.4. Owing to the alkaline reaction in the cathode chamber, the cations of insoluble bases must be precipitated as soon as they reach this chamber.

In this experiment no definite end point was apparent in the yield of electrodialyzable material. Subsequent tests showed, however, that when the electrodialysis of the Sharkey soil was prolonged to 42 hours, titrable bases in the hourly extract were less than 0.01 milliequivalent and the yield of insoluble bases had practically vanished. This is a fairly definite end point, although it is approached asymptotically. The soil colloids in the following experiments were therefore electrodialyzed until the hourly cathode extract required less than one drop of N/10 acid for neutralization and contained little or no sediment.

It will be noted that the yield of acids in this experiment was only one-eighth to one-ninth that of the soluble bases. The titrable acid consisted chiefly of H_2SO_4 and H_3PO_4 .

While Table 2 shows the rate at which the combined soluble bases are removed from the whole soil, it does not show the rates at which the individual bases are removed from the colloidal material alone. In order to gain information on this point, a sample of the Sharkey colloidal material was electrodialyzed and three successive fractions of the cathode dialysate were analyzed separately.

The Sharkey colloidal material electrodialyzed was not part of the sample, the analysis of which is given in Table 1, but was isolated from the same lot of soil and was very similar in composition. A sample of 54.6 gm. was electrodialyzed for 42 hours, the cathode solution being removed hourly. The last cathode solution required only 1 drop of N/10 acid for neutralization and was about free from sediment. The cathode solutions removed each hour were combined to form three separate fractions that corresponded to the changes observed in the preliminary experiment. The first fraction, A, amounting to 1,850 c. c., contained the first 12 hourly solutions, all of which were clear. Fraction B, of 930 c. c., included the solutions obtained during the 6 succeeding hours and represented the solutions which contained the light-colored sediment. Fraction C, of 3,060 c. c., collected in the last 24 hours, contained all solutions showing an appreciable quantity of brownish sediment.

Table 3 shows the bases present in each fraction, expressed as percentage of the weight of oven-dry colloid.

TABLE 3.—*Successive fractions of bases removed by electro dialysis of the Sharkey soil colloid*

Fraction	Fe_2O_3	Al_2O_3	TiO_2	MnO	CaO	MgO	K_2O	Na_2O
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
A.....	Trace.	Trace.	None.	Trace.	1.075	0.007	0.091	0.030
B.....	0.008	0.054	None.	0.003	.137	.136	.017	.009
C.....	.091	.109	None.	.016	.058	.245	.022	.007
Total.....	.099	.163	None.	.019	1.270	.388	.130	.046

The first or clear fraction contained most of the lime, potash, and soda removed during the whole period of electro dialysis; only a small quantity of magnesia and traces of the other bases were present. In the second fraction magnesia increased markedly, and alumina, iron, and manganese began to appear in determinable quantities. The last fraction differed from the second in a further increase in the magnesia, iron, alumina, and manganese. The order in which the different bases appear in the cathode chamber is about as follows: Ca, K, and Na; Mg; Al, Mn, and Fe.

This order may not represent the order in which the cations are actually released from the soil colloid particles. The maintenance of Fe, Al, Mn, and Mg in solution and consequently their transport by the current is dependent on the P_H . Under the conditions of the experiment, the P_H in the central chamber is modified by the transport of H and OH ions resulting from the electrolysis of acids and bases in the anode and cathode chambers, respectively. At the beginning

of the experiment the layer in the central chamber adjacent to the cathode parchment is alkaline and the layer adjacent to the anode parchment is acid. Later, as the bases are removed, the reaction becomes acid, throughout the chamber.

PART OF THE BASES IN THE COLLOID REMOVED BY ELECTRODIALYSIS

In order to determine what part of the bases in the soil colloid are removable by the action of the current, samples of the Sharkey and Norfolk soil colloids which had previously been analyzed (Table 1) were electrodialyzed until the hourly cathode dialysate was nearly clear and neutral.

Table 4 shows the quantity of each base removed by electrodialysis, as percentage of the sample; the quantity of each base present, as percentage of the sample; and the proportion of each base removed, as percentage of the quantity present.

TABLE 4.—*Proportion of each base removed by electrodialysis*^a

Kind of colloid	Determinations	Al ₂ O ₃	Fe ₂ O ₃	MnO	CaO	MgO	K ₂ O	Na ₂ O	NH ₂
		<i>Per cent</i> 0.29	<i>Per cent</i> 0.14	<i>Per cent</i> 0.017	<i>Per cent</i> 1.33	<i>Per cent</i> 0.37	<i>Per cent</i> 0.16	<i>Per cent</i> 0.04	<i>Per cent</i> 0.12
Sharkey	Base removed (as percentage of sample).	22.52	8.12	.035	1.36	2.52	1.89	.21	-----
	Total base present (as percentage of sample).	1.29	1.72	48.571	97.79	14.68	8.47	19.05	-----
	Proportion of base removed (as percentage of total base present).	.20	.03	.002	.16	.05	.06	.01	.18
Norfolk	Base removed (as percentage of sample).	33.51	11.24	.005	.30	.44	.49	.25	-----
	Total base present (as percentage of sample).	.60	.27	40.000	53.33	11.36	12.24	4.00	-----
	Proportion of base removed (as percentage of total base present).								

^a Most of the ammonia present was adsorbed by the colloid in the course of its separation from the soil.

It is apparent that the total quantity of bases removable by electrodialysis may vary greatly with different colloids. In the case of the Sharkey colloid the sum of the bases removed amounts to 2.47 per cent of the sample, while in the case of the Norfolk colloid the yield of bases is only 0.69 per cent, or less than one-third as much.

The percentage of each base present which is removed varies less widely for the two colloids than the total quantity removed. With the possible exception of potash, the proportion of base removed to quantity present is greater in the case of the Sharkey colloid than in the case of the Norfolk. This is true even for iron and aluminum, although the Norfolk contains considerably more of these bases. In general, however, the proportion of calcium, magnesium, or manganese removed by electrodialysis is not very different for the two colloids, considering the widely different quantities of these constituents in the two materials.

While the proportion of each base removed is not exactly the same for the two colloids, it is evident that the various bases are characterized by different degrees of removability. The proportion of the lime or manganese and the proportion of alumina and iron

removable is still less. These differences in the comparative removability of the bases probably hold for the colloids of most humid soils, since the Sharkey and Norfolk colloids are representative of widely different groups. Colloids from alkali or salt soils, may, however, show a greater proportion of the sodium replaceable.

MATERIAL REMOVED BY ELECTRODIALYSIS COMPARED WITH THAT REMOVED BY BASE EXCHANGE

It is quite apparent from the work of other investigators on base exchange that various salt solutions extract a fairly definite quantity of bases from the colloidal soil material. A comparison of the bases removed by electrodialysis with those removed by methods in use on base exchange was desirable, since both procedures yield a fairly definite quantity of bases.

As the reagents most commonly used at the present time for determining the exchangeable bases in soils are normal NH_4Cl and 0.05 normal HCl , the exchangeable bases in the Sharkey and Norfolk colloid were determined by these solutions, following the standard procedure described on page 554. The colloidal materials used were portions of the lots that were prepared for the work on electrodialysis.

Table 5 shows the quantities of bases and acids removed from the two colloids by the NH_4Cl and HCl solutions compared with the quantities removed by electrodialysis. The quantities are expressed as percentages of the weight of oven-dried colloid.

TABLE 5.—Bases and acids extracted from colloidal soil materials by base exchange reagents and by electrodialysis

Kind of colloid	Treatment of colloid	SiO_2	Fe_2O_3	Al_2O_3	MnO	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3
		<i>Per ct.</i> None.	<i>Per ct.</i> Trace.	<i>Per ct.</i> Trace.	<i>Per ct.</i> 0.016	<i>Per ct.</i> 1.33	<i>Per ct.</i> 0.36	<i>Per ct.</i> 0.13	<i>Per ct.</i> 0.03	<i>Per ct.</i> Trace.	<i>Per ct.</i> (*)
Sharkey.	Extraction with N/1 NH_4Cl .										
	Extraction with N/20 HCl .	0.14	0.35	0.80	.019	1.39	.41	.14	.03	0.06	0.04
	Electrodialysis.....	.05	.14	.29	.017	1.33	.37	.16	.04	.04	.04
Norfolk..	Extraction with N/1 NH_4Cl .	None.	Trace.	Trace.	.002	.15	.04	.07	.02	Trace.	(*)
	Extraction with N/20 HCl .	0.09	0.05	0.52	.002	.16	.03	.08	.01	0.01	0.04
	Electrodialysis.....	.02	.03	.20	.002	.16	.05	.06	.01	.01	.04

* Not determined.

It is apparent that base exchange methods and electrodialysis remove approximately the same quantities of constituents from the soil colloids. The agreement between the methods is very close, except in the case of alumina, iron, and silica. The greater quantities of these constituents removed by HCl and by electrodialysis than by NH_4Cl is probably conditioned by the P_H of the solution in contact with the colloid. The P_H of the total NH_4Cl extract of the Sharkey colloid was 6.2 and that of the Norfolk 6.1; the P_H of the electrodialyzed colloids was about 5.0; while the P_H of the final HCl extract may be assumed to be the same as that of the N/20 HCl solution, about 1.5. Doubtless the NH_4Cl treatment would have removed some Al and Fe from the Norfolk colloid in its natural condition. The Norfolk colloid is naturally acid; but in isolating

this colloid from the soil a small amount of ammonia was used to promote dispersion and this was apparently sufficient to render the colloid nearly neutral.

In view of the practical identity of the material removed by the different procedures, the conclusions regarding the material removed by electrodialysis also apply to the material removed by base exchange. Briefly, the total quantity of bases removed by base exchange from the two colloids varies widely; the part of each base removed is not the same for the two colloids, but it is sufficiently similar to justify the conclusion that in most colloids lime and manganese are removed to a greater extent than magnesia, potash, or soda, and that a still smaller part of the alumina and iron are removable.

The proportion of one base to another in the materials extracted by electrodialysis and by base exchange is also of interest. This is shown in Table 6 for the monovalent and divalent bases. Iron and aluminum were omitted in this calculation, since such different quantities were extracted by the various methods that the inclusion of these bases would have obscured the relationship that obtains between the other bases. Ammonia was determined only in the electrodialysates and is therefore also omitted.

Table 6 gives the total milliequivalents of Ca, Mg, K, Na, and Mn extracted from 1 gm. of colloid by electrodialysis and by base exchange reagents and the proportion of each base in the total. The bases exchanged with methylene blue, which are included in this table, were determined in connection with another investigation and do not represent the maximum quantity that can be displaced by this reagent, since only a small quantity of the dye was used.

TABLE 6.—Proportions between the bases in material removed from the colloids by electrodialysis and by base exchange

Kind of colloid	Treatment	Mn, Ca, Mg, K, and Na extracted per gm. colloid	In 100 milliequivalents were found—				
			Mn	Ca	Mg	K	Na
		<i>M. eq.</i>	<i>M. eq.</i>	<i>M. eq.</i>	<i>M. eq.</i>	<i>M. eq.</i>	<i>M. eq.</i>
Sharkey...	Electrodialysis.....	0.712	0.67	66.7	26.0	4.8	1.8
	Displaced by N/1 NH ₄ Cl.....	.698	.66	68.1	25.8	4.1	1.4
	Displaced by n. 0.05 HCl.....	.746	.72	66.5	27.2	4.2	1.3
	Displaced by methylene blue.....	.486	^b N. D.	65.5	27.6	4.9	2.0
	Electrodialysis.....	.099	0.61	57.6	25.2	13.1	3.3
Norfolk...	Displaced by N/1 NH ₄ Cl.....	.096	.62	56.3	20.9	15.6	6.3
	Displaced by n. 0.05 HCl.....	.093	.65	61.2	16.1	18.3	3.3
	Displaced by methylene blue.....	.061	^b N. D.	58.3	20.5	16.4	4.9

^a Mn not included.

^b Not determined.

It is evident that the proportions between the various monovalent and divalent bases are practically identical in the material removed by the four different methods of extraction within the limits of experimental error.

The proportions between the bases in the Sharkey extract are somewhat different from the proportions in the Norfolk extract. It will be noted, however, that the two colloids differ less widely in the proportions between the bases extracted than in the total quantity

removed or than in the proportions between removable and non-removable bases shown in Table 4.

The proportions between the exchangeable bases in the two colloids are about the same as those reported by other investigators. Apparently these proportions hold approximately for such soils. This is perhaps not surprising, since the river waters of humid regions, such as the Atlantic slopes of North America and western and central Europe, contain about the same equivalent proportions of the monovalent and divalent cations (5, p. 75, 97).

DISPLACEMENT OF HYDROGEN FROM ELECTRODIALYZED COLLOIDS

It has repeatedly been shown that when the exchangeable bases in a soil are displaced by the cations of a salt, an equivalent quantity of the displacing ions are retained by the soil. Since the material removed by electrodialysis was identical with that removed by the neutral salt, except for iron and aluminum, it seemed possible that in electrodialysis there might also be an exchange of cations. Furthermore, the fact that in cataphoresis determinations the electrodialyzed colloid behaved in a manner similar to that of the original colloid, showed that it must contain some adsorbed cations. The only cations available to take the place of the cations removed by electrodialysis would be the hydrogen ions of the water.

If hydrogen were adsorbed by the colloid on electrodialysis, it should appear as acid when the material is treated with a neutral salt solution. The Sharkey colloid was therefore leached with 2 liters of a hot four normal KCl solution and the filtrate titrated with tenth normal NaOH, brom thymol blue being used as an indicator. Five grams of the colloid yielded 3.28 milliequivalents of acid to the first liter and only 0.03 milliequivalent to each of the third and fourth half liters. A normal CaCl_2 solution gave almost identical results. It is thus evident that neutral salts displace a fairly definite quantity of acidity from the electrodialyzed colloid. The quantities of acid displaced from the Sharkey and Norfolk colloids by 1 liter of normal CaCl_2 are given in Table 7, calculated as milliequivalent of hydrogen ion per gram of colloid.

After treatment with the CaCl_2 solution, the two colloids were washed free from chlorides and again electrodialyzed to determine how much Ca had been adsorbed in displacing the hydrogen from the electrodialyzed material. The quantities of Ca removed by the second electrodialysis are also given in Table 7.

It will be seen that in the case of the Sharkey colloid the Ca removed by the second electrodialysis was almost exactly equivalent to the acidity produced by the CaCl_2 treatment, while in the case of the Norfolk colloid, the Ca removed was slightly greater than the acidity. Evidently the electrodialyzed colloid gives the same type of exchange reaction with neutral salts as the untreated soil or colloid, the only difference being that the electrodialyzed material exchanges hydrogen for the cation of the salt instead of a mixture of monovalent and divalent bases or a mixture of these bases and hydrogen. In the case of the electrodialyzed Sharkey colloid, the exchange capacity is shown by either the acidity liberated or by the Ca adsorbed; but in the case of the Norfolk colloid the Ca adsorbed is probably a more

accurate measure of the exchange capacity than the acidity developed. Work in progress indicates that the discrepancy of 0.036 milliequivalent between acidity developed by CaCl_2 treatment and Ca adsorbed is probably due to an adsorption of Cl ions by the electrodyalized Norfolk colloid.

In Table 7 the exchange capacity of the electrodyalized colloids is compared with that of the untreated colloid as measured by the monovalent and divalent cations removed in electrodyalisis and as determined by the Ca adsorbed from neutral CaCl_2 .⁵

TABLE 7.—*Exchange capacities of the Sharkey and Norfolk soil colloids before and after electrodyalisis*

Determination	Milliequivalent per gram of colloid	
	Sharkey	Norfolk
Ca adsorbed from neutral CaCl_2 by the original colloids.....	0.796	0.207
Total monovalent and divalent cations removed by electrodyalisis.....	.785	.208
Hydrogen displaced from electrodyalized colloids by CaCl_2 solution.....	.640	.164
Ca adsorbed by electrodyalized colloids from the CaCl_2 solution, determined by a second electrodyalisis.....	.647	.201

It will be seen that the exchange capacities of both colloids are almost the same when measured by Ca adsorbed as when measured by monovalent and divalent bases removed by electrodyalisis. The exchange capacity of the electrodyalized Norfolk colloid is almost identical with that of the untreated material. The exchange capacity of the electrodyalized Sharkey, however, is about 20 per cent less than that of the original colloid. The results on the whole tend to substantiate the idea that in the process of electrodyalisis there is an exchange of hydrogen for all, or most, of the bases removed and the adsorbed hydrogen can in turn be replaced by other cations.

In the preceding considerations of the exchange capacity of the electrodyalized colloids, the Fe and Al were not regarded as constituting part of the exchangeable cations, although the CaCl_2 treatment yielded quantities of the trivalent cations in addition to those already removed by electrodyalisis. The electrodyalized Sharkey yielded 0.458 milliequivalent of combined Al and Fe, 0.353 milliequivalent of which was Al, and the Norfolk colloid gave 0.353 milliequivalent of the trivalent cations, 0.124 milliequivalent being Al. If Fe and Al did exchange with the Ca of the CaCl_2 solution, they would of course be hydrolyzed and produce acidity; so both acid and trivalent bases should not be included in calculating the exchange capacity. Inasmuch as the combined Fe and Al in the CaCl_2 filtrate was sufficient to account for only approximately two-thirds of the acidity, the acid was apparently the fairer measure of the exchange capacity, even if part (or all of it) resulted from exchanged Fe and Al.

On the whole, it appears as though electrodyalisis may be looked upon as a form of base exchange, in which hydrogen ions are substituted for monovalent and divalent bases. While by the usual methods of base exchange in the soil we continually introduce a high

⁵ The Ca adsorbed after prolonged leaching of the colloid with neutral CaCl_2 solution was determined by subsequent displacement with NH_4Cl .

concentration of the displacing cation and continually withdraw the displaced ions, we arrive in electrodialysis at the same result by the mere withdrawal of the cations, in which case the displacing ion must be the ever-present hydrogen ion of the water. Both methods are in principle the same: A displacement or equilibria either by increasing one factor or by decreasing the other; in one case we apply a push, in the other a pull.

CONDITION OF THE BASES REMOVED BY ELECTRODIALYSIS OR BASE EXCHANGE

It was shown (Table 4) that electrodialysis removed about half the manganese present in the two colloids, most of the calcium, and only a small part of the magnesium, potassium, and sodium. A neutral salt removed almost identical proportions of these bases (Table 5). It is thus apparent that part of each of the monovalent and divalent bases is more reactive than the remainder. This would indicate that each of the monovalent and divalent bases in the colloid is present in two conditions. The condition of the nonreactive part of the bases might be described simply by the facts as nonexchangeable, in the absence of definite information regarding the ultimate chemical constitution of the insoluble part of the soil colloid particle.

The exchangeable bases are usually regarded as being present in the adsorbed condition. From the rapidity of base exchange reaction, Hissink (13) concludes that the exchangeable bases are situated on the surface of the soil particles in the adsorbed condition and ascribes the charge of the particles to a partial dissociation resulting in a Helmholtz double layer. Wiegner (21) identifies the exchangeable cations with the cations of the outer Helmholtz layer. In fact he calculates what size the primary particles in a clay having an exchange capacity of 3.5 milliequivalents per gram would have to be in order to accommodate all the exchangeable cations in the outer layer.

Wiegner arrives at a value of $7.29 \mu\mu$ for the diameter of the primary particles which would afford the required surface. However, an error occurs in his calculations in that he assumes the surface per gram of clay varies inversely as the square of the radius instead of inversely as the radius.⁶ If this error is corrected, the diameter of the hypothetical primary particles becomes $0.106 \mu\mu$, or about that of a hydrogen ion. This is obviously absurd.

In a previous publication it was shown that there is an intimate relation between the exchangeable bases and the electrokinetic potential of soil colloids (18). Although the exchangeable cations influenced the charge of the particles, it appeared quite improbable that all the exchangeable cations were present in the outer Helmholtz layer. The fact that on neutralizing the charge of the particles with methylene blue the cataphoretic movement was not markedly reduced until most of the exchangeable cations had been replaced indicated that only a part of the cations were dissociated.

From the formula connecting the charge on a particle with the electrokinetic potential, applied by Von Hevesy (12), Freundlich (6, p. 538), and others to colloidal particles in general and by Wiegner

⁶ The surface presented by 1 gm. of colloid is equal to the surface of one particle, $4\pi r^2$, multiplied by the number of particles per gram $\frac{1}{4\pi r^2 \times \text{sp. gr.}}$; hence surface = $\frac{3}{r \times \text{sp. gr.}}$

to clay particles, the number of electronic charges on a particle of the Sharkey colloid has been calculated, using the values for the electrokinetic potential and the radius of the particles reported in previous publications (2) and assuming a thickness of the double layer of $5 \mu\mu$.⁷ The number of charges in terms of univalent ions or electrons on a particle of the Sharkey colloid was thus found to be 857 ions.

The exchange capacity of the Sharkey colloid at the point of neutrality is about 0.8 of a milliequivalent. This is equal to $4.8 \times 10^{+20}$ ions. Since 1 gm. of the colloid would contain $9.6 \times 10^{+14}$ particles with a radius of $45.5 \mu\mu$, the number of exchangeable cations per particle is 505,208, or about 590 times the number of cations that should, according to the above calculation, be present in the outer, electropositive layer surrounding the particle. If, therefore, the assumed thickness of the double layer and the size of the particle determined by ultramicroscopic count represent the true values, it is evident that only a very small fraction of the exchangeable bases exist in the dissociated condition.

A thickness of the double layer of $5 \mu\mu$ is probably too low rather than too high, since the cataphoretic movement was measured in distilled water, under which conditions Gouy (11) assumes a much greater thickness. But even if a thickness of the double layer of molecular dimensions were assumed, the charge on the particles would not nearly account for all the exchangeable bases.

It is of course probable that the ultramicroscopic particles of the colloid consist of aggregates of smaller, so-called primary particles. However, the primary particles would have to be $0.077 \mu\mu$ in radius to afford sufficient surface for 0.8 milliequivalent of ions, if the number of ions per unit surface is assumed to be the same as was calculated for the particle with $45 \mu\mu$ radius. This value is absurd. Hence, if the theoretical deductions involved in the formulas connecting electrical migration with electrokinetic potential and charge of the particles are correct, it would appear that only a part of the exchangeable bases are present in the dissociated condition.

SUMMARY

Two soil colloids, which are representative of widely different groups of colloidal soil materials, were electrodialyzed in order to determine what part of the bases can be removed by this process. The quantities of bases removed by electrodialysis were compared with the quantities removed by methods commonly used for determining the exchangeable bases in soils.

Preliminary work showed that the quantity of bases that can be removed by electrodialysis is fairly definite and that the order in which the different bases appear in the cathode chamber is about as follows: Ca, K, and Na; Mg; Al, Mn, and Fe. It is pointed out, however, that this may not represent the order in which the cations

⁷ The charge, e , on a colloidal particle = $\frac{\epsilon D r}{\delta} (\psi + \delta)$, where ϵ is the electrokinetic potential, D the dielectric constant of the dispersion medium, r the radius of the particle and δ the thickness of the double layer. In the case of the Sharkey colloid, $\epsilon = 33$ millivolts or $\frac{.033}{300} = 1.1 \times 10^{-4}$ electrostatic unit, and $r = 4.55 \times 10^{-6}$ cm. D is taken as ≈ 81 for water and δ is assumed to be $5 \mu\mu$, or 5×10^{-7} cm., the value generally assumed. The charge for the Sharkey particle becomes 4.69×10^{-7} electrostatic unit. Since one ion has 4.77×10^{-10} electrostatic unit, the charge on the Sharkey particle would be that of 857 ions.

are released from the colloid particles, since the appearance of the bases in the cathode chamber is largely affected by the hydrogen-ion concentration in the compartment containing the colloid.

The total quantity of bases that electro dialysis removed from one colloid was about five times that removed from the other. While the percentages of the Ca or Na in the colloid that are removable do not agree closely in the case of the two colloids, it is evident that the various bases are characterized by different degrees of removability. The proportion of the total lime or manganese removable by electro dialysis is much greater than the proportion of magnesium, potassium, or sodium, and the proportion of the total aluminum or iron removable is still less.

Extraction of the two colloids with normal NH_4Cl or $\frac{N}{20}\text{HCl}$ yields quantities of the monovalent and divalent bases that are almost identical with those obtained by electro dialysis.

Treatment of the electro dialyzed colloids with a CaCl_2 solution develops quantities of acidity that approximate the base exchange capacities of the untreated colloids. It appears that in the process of electro dialysis there is a substitution of hydrogen ions from the water for most of the monovalent and divalent cations removed by the electric current.

It is evident that each of the monovalent and divalent bases in the colloid is present in two conditions which might be defined simply as exchangeable and nonexchangeable.

The quantity of the exchangeable bases that can exist as cations in an outer Helmholtz layer surrounding the particle is considered. It is pointed out that if the deductions involved in formulas connecting electrical migration with electrokinetic potential and charge of the particles are correct, only a part of the exchangeable bases in the colloid are present in the dissociated condition.

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A CHEMICAL AND PHYSIOLOGICAL STUDY OF MATURITY IN POTATOES¹

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INTRODUCTION

The problem of maturity in potatoes assumes considerable practical importance in connection with several claims for the superiority of immature potatoes for seed. The term "immature seed" expresses a rather vague idea on account of our lack of a definite and scientific conception of what constitutes maturity in potatoes.

One object of the experiments reported in this paper was to determine the character of the chemical changes in potatoes while they are ripening and maturing on the vines and to correlate, if possible, the different stages of maturity with certain characters of the vines. The chief information sought was the extent to which the chief maturing processes in the tubers may continue in storage. Will potatoes apparently immature but large enough for seed attain during storage the percentage composition of solids and the physiological condition characteristic of tubers allowed to fully mature on the vine? The nitrogenous constituents are specially emphasized in this paper.

EXPERIMENTAL PROCEDURE

The seed used for the experimental crop was Irish Cobbler grown in Garrett County, Md. At intervals, beginning when the vines were in bloom, four to six typical hills were dug and the tubers divided into two lots matched for size and number of tubers. One lot was sampled immediately for analysis and the other was placed in cellar storage at a fairly constant temperature of 68° F. The storage lots were all sampled for analysis at the same time; that is, at the end of the rest period on October 26.

The dates on which the tubers were dug and the condition of the plants on each of these dates were as follows:

- June 17, plants in full blossom.
- June 24, blossoms gone, but leaves green.
- July 8, tips of leaves beginning to die; vines prone.
- July 15, about 80 per cent of the leaves dead.
- July 22, leaves all dead; vines partly dead.
- August 27, vines brown and dry.

The average number and weight of the tubers per hill and the percentage of loss in weight of the different lots during the storage period are given in Table 1.

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TABLE 1.—Average number and weight of tubers per hill on the different digging dates, and loss in weight of the different lots during the storage period

Date of digging	Average number of different sized tubers per hill					Total number	Total weight per hill in ounces	Percentage loss in weight on Oct. 26
	Pea	Marble	Walnut	Egg	Larger			
June 17.....	9.3	2.3	4.2	1.9	0	17.6	5.5	16.45
June 24.....	10.2	3.0	2.6	3.0	0	18.8	7.3	12.38
July 1.....	8.6	4.3	2.0	3.3	2.6	21.0	20.6	8.67
July 15.....	8.0	1.5	3.5	1.5	8.0	22.5	26.1	6.70
July 22.....	2.5	1.6	3.0	3.0	5.6	15.6	28.0	6.10
Aug. 27.....	2.0	1.6	4.6	3.3	4.3	16.0	26.8	4.62

ANALYTICAL METHODS

SAMPLING AND DETERMINATION OF MOISTURE

The tubers were ground to a pulp in a meat grinder, and after thorough mixing samples of the fresh pulp were weighed out for moisture, sugars, and nitrogenous constituents.

To determine the moisture, about 5 gm. of pulp were dried to constant weight at 70° C. in a vacuum of 30 inches of mercury.

SUGARS

For the determination of sugars a sample of 50 gm. was weighed into a counter-poised Kohlrausch sugar flask into which had been placed 0.25 gm. of CaCl_2 . The pulp was covered immediately with 75 c. c. of 95 per cent boiling alcohol and the mixture brought to boiling on the steam bath, after which sufficient hot water was added to bring the extraction medium to 50 per cent alcohol by volume. After boiling five minutes the samples were allowed to stand overnight in the 50 per cent alcohol and the volume then completed to the mark with 95 per cent alcohol and stored. After the alcohol had been removed the sugars were determined in the aqueous solution according to the Munson and Walker method.

ASH, STARCH, AND CRUDE FIBER, TOTAL NITROGEN

The ash was determined by igniting the residues from the moisture determinations. The percentage of ash in the original moist sample was calculated by indirect proportion.

The starch and crude fiber were determined by the official methods of the Association of Agricultural Chemists, using oven-dried material. The diastase method with subsequent acid hydrolysis was employed for starch.

From 8 to 9 gm. of fresh pulp were weighed from a weighing bottle with ground-in glass stopper and the total nitrogen determined by the official Gunning method.

DISTRIBUTION OF NONPROTEIN NITROGEN

The possibility of autolytic changes is always present during aqueous extractions of the soluble nonprotein nitrogenous constituents in fresh plant tissues. This objection could be eliminated by using alcohol of sufficient strength to prevent autolytic changes if complete extraction of these constituents is possible in such a medium. Bacterial action and filtering difficulties would also be largely avoided.

Jodidi ³ extracted the amino acids of rye flour directly with boiling 92 per cent alcohol. He found an average of 20.43 per cent mono-amino nitrogen in one variety and 35.07 per cent in another variety when calculated on the basis of alcohol-soluble nitrogen. These percentages were higher than those found in the aqueous extracts of the same varieties, based upon water-soluble nitrogen. However, he does not state the relative amounts of either total soluble nitrogen or total nonprotein nitrogen obtained by the water and alcohol extractions in this experiment. In a previous experiment he found that water extracted about three times as much nitrogen as did the alcohol. He states that this is perhaps due to the greater proportion of protein matter taken up by the water. The weight of the sample used for the alcohol extraction was four times that for the water extraction. Then, too, all of the nonprotein nitrogen may not be soluble in the strong 92 per cent alcohol used for the extractions.

Osborne, Wakeman, and Leavenworth ⁴ precipitated the protein of alfalfa juice with alcohol of 53 per cent by weight. They assume that the nonprotein nitrogen is soluble in this percentage of alcohol, although they state that their evidence is not entirely convincing to show that a small part of the precipitate may not belong to nonprotein substances insoluble in 53 per cent alcohol. In later work by Vickery ⁵ the nonprotein nitrogenous constituents of the juice of the alfalfa plant were determined in the filtrate from a 53 per cent alcoholic solution.

Preliminary experiments were conducted to test the effectiveness of alcoholic extractions as compared with aqueous extractions of the soluble nonprotein nitrogenous constituents in potatoes. The results in Table 2 are based upon the following detailed procedures for both types of extractions:

TABLE 2.—Percentages of nonprotein nitrogen in potatoes obtained by water and alcoholic extractions

Sample No.	Water extract	Alcoholic extract
1	0.226	0.217
2	.227	.218

The tubers were cooled and then grated on a nutmeg grater in a mortar surrounded by cracked ice. By this means the pulp was maintained at a temperature of 4° C. during sampling.

For the water extractions, samples of pulp of 100 gm. each were stirred with 100 c. c. of water and strained through a small coffee strainer. By gently pressing the pulp with a spatula, most of the starch and cell colloids, which make filtering of the pulp difficult, were removed. The pulp was transferred with 200 c. c. of water to a smooth filter in a Büchner funnel. Suction was applied after the addition of each portion of water. The filtrate and strained juice

³ JODIDI, S. L., and WANGLER, J. G. PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON CEREALS. IV. ON THE PRESENCE OF AMINO ACIDS AND POLYPEPTIDES IN THE UNGERMATED RYE KERNEL. *Jour. Agr. Research* 30: 989-994. 1925.

⁴ OSBORNE, T. B., WAKEMAN, A. J., and LEAVENWORTH, C. S. THE WATER-SOLUBLE CONSTITUENTS OF THE ALFALFA PLANT. *Jour. Biol. Chem.* 53: 411-429. 1922.

⁵ VICKERY, H. B. SOME NITROGENOUS CONSTITUENTS OF THE JUICE OF THE ALFALFA PLANT. I. THE AMIDE AND AMINO ACID NITROGEN. *Jour. Biol. Chem.* 60: 647-655. 1924.

were combined and sufficient 95 per cent alcohol added to make 53 per cent by weight. The pulp was dried at 70° C. and ground to pass a 40-mesh sieve. The material which weighed about 20 gm. was extracted with 300 c. c. of ammonia-free water by agitating on a mechanical shaker for two hours. Toluol was used as an antiseptic. The material was filtered in a Büchner funnel and thoroughly washed. Alcohol was then added to the filtrate to make 53 per cent by weight. This was combined with the first alcoholic solution. A flocculent precipitate which appeared upon the addition of alcohol was filtered off through paper pulp in a Büchner filter. The alcohol was added to preserve the extracts and also to test the completeness with which this percentage of alcohol would precipitate the soluble protein in a potato extract. Osborne⁶ claims that this percentage of alcohol will effect complete precipitation of the soluble protein from an aqueous extract of alfalfa. In these potato extracts the precipitation was far from complete, as shown by the heavy precipitate obtained later by lead acetate.

Similar samples of pulp of 100 gm. each were used for the alcohol extractions. The samples were covered immediately with 400 c. c. of boiling 95 per cent alcohol and stored for one week. The final concentration of the storage alcohol was approximately 75 per cent. The flasks were shaken occasionally during the storage period.

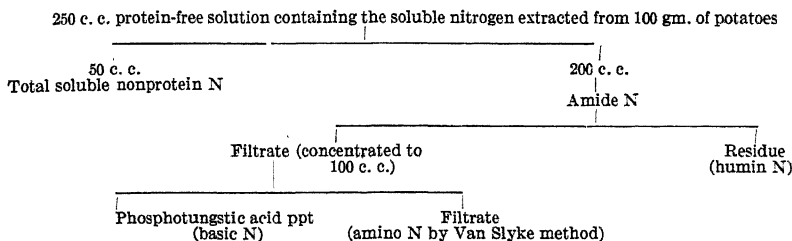
The storage alcohol was filtered through a smooth filter paper in a Büchner funnel and the pulp transferred to the funnel, 50 c. c. of 50 per cent alcohol being used to wash out the flask. After as much of the alcohol was removed as was possible by suction, the pulp was dried in the funnel at 70° C. and ground to pass a 40-mesh sieve. It was then transferred to the original flask and covered with 200 c. c. of hot 50 per cent alcohol by volume. The flask was shaken several times during a period of 24 hours, after which the alcohol was decanted on the same filter and another 200 c. c. of hot alcohol of the same strength added to the flask. After the flask had been shaken frequently for a period of two hours the entire contents were transferred to the filter, and 100 c. c. of 50 per cent alcohol was used to wash out the flask. Suction was applied after the addition of each portion of alcohol. A third extraction with 50 per cent alcohol did not remove any additional nitrogen.

Both the alcoholic and aqueous extracts were concentrated on the steam bath and the soluble protein precipitated with basic lead acetate. The excess of lead was removed by hydrogen sulphide and the final filtrates were made up to 500 c. c. The total nitrogen was determined in 100 c. c. aliquots.

Since the final procedure for alcoholic extraction yielded practically the same amounts of nonprotein nitrogen as the aqueous extractions, it was adopted for the analyses of the research material. The only deviation from this procedure was the period of storage in the 75 per cent alcohol. This period was much longer than that of the preliminary experiments. The total amount of nonprotein nitrogen found in the preliminary experiments was larger than the maximum found in the material under investigation, but the material was not comparable since much older potatoes of a different crop were used

⁶ OSBORNE, T. B., WAKEMAN, A. J., and LEAVENWORTH, C. S. THE WATER-SOLUBLE CONSTITUENTS OF THE ALFALFA PLANT. *Jour. Biol. Chem.* 53: 411-429, 1922.

in the preliminary experiments. The following graphic scheme will serve to indicate the nitrogen fractions determined in the protein-free solutions.



The 200 c. c. aliquot of the protein-free solution was acidified with 20 c. c. of concentrated HCl and boiled under a reflux condenser for two hours. The acid was removed by evaporating the solution almost to dryness on a water bath. Calcium hydroxide was used for the distillation of the ammonia from the hydrolyzed acid amides. The clear supernatant liquid above a 10 per cent suspension of calcium hydroxide furnished sufficient alkali without diluting the solution to too great a volume. An excess of calcium hydroxide interfered with subsequent digestions. Duplicate determinations were made in which reduced pressure was used for both the removal of the HCl and the distillation of the ammonia, but this precaution was found to be unnecessary with these solutions.

The basic nitrogenous constituents in the filtrate from the humin nitrogen residue were removed according to the usual procedure with phosphotungstic acid. This reagent also precipitates any proteoses or peptone that may be present.

The phosphotungstic acid precipitate was washed with a solution containing 2.5 per cent phosphotungstic acid and 3.5 per cent HCl and cooled to 0° C. in order to avoid as far as possible the danger of dissolving any of the phosphotungstates of the hexone bases. The phosphotungstic acid precipitate was oxidized by the Kjeldahl method without removing the phosphotungstic acid, as it was found that this did not interfere if the digestion was continued for three hours after the solution is clear.

The combined filtrate and washings from the phosphotungstic precipitate was just neutralized with NaOH, cleared with a little acetic acid and concentrated to 100 c. c. Aliquots of 10 c. c. were used to determine amino nitrogen by the Van Slyke micromethod.

ANALYTICAL RESULTS

The data obtained from all of the analytical work are tabulated in Tables 3 to 9. The distribution of nitrogen is also shown graphically in Figure 1.

TABLE 3.—Percentage composition of potatoes dug at different stages of development and analyzed immediately

Date of digging	Moisture	Ash	Crude fiber	Proteins (N x 6.25)	Total sugars	Starch
June 17.....	83.66	0.91	0.34	1.77	1.03	10.57
June 24.....	82.72	.91	.30	1.85	1.03	11.47
July 8.....	80.80	.91	.32	1.82	.68	13.80
July 15.....	79.35	1.00	.31	2.23	.30	14.98
July 22.....	80.20	1.02	.31	2.24	.19	14.25
Aug. 27.....	81.02	1.03	.32	2.17	.26	13.60

TABLE 4.—Percentage composition of potatoes dug at different stages of development and analyzed at the end of the rest period, October 26

Date of digging	Moisture	Ash	Crude fiber	Proteins	Total sugars	Starch
June 17.....	81.26	1.20	0.47	2.00	0.41	10.82
June 24.....	81.78	1.10	.43	1.88	.41	11.68
July 8.....	80.09	1.05	.37	1.93	.31	13.34
July 15.....	78.33	1.05	.37	2.18	.24	14.76
July 22.....	79.56	1.06	.35	2.33	.19	14.23
Aug. 27.....	79.65	1.11	.33	2.28	.21	15.05

TABLE 5.—Percentage composition of Irish Cobbler potatoes dug at different stages of development and analyzed at the end of the rest period

[Results calculated on basis of moisture content at time of digging]

Date of digging	Ash	Crude fiber	Proteins	Total sugars	Starch
June 17.....	1.05	0.41	1.75	0.36	9.44
June 24.....	1.05	.40	1.79	.39	11.08
July 8.....	1.02	.36	1.86	.30	12.87
July 15.....	1.00	.35	2.08	.23	14.16
July 22.....	1.03	.34	2.26	.19	13.78
Aug. 27.....	1.03	.31	2.13	.20	14.03

TABLE 6.—Percentage of sugars in Irish Cobbler potatoes at different stages of development and at the end of the rest period

Date of digging	Percentage of sugars in potatoes—					
	At time of digging			At end of rest period		
	Reducing sugars	Sucrose	Ratio	Reducing sugars	Sucrose	Ratio
June 17.....	0.203	0.788	3.88	0.273	0.138	0.50
June 24.....	.146	.804	6.19	.278	.122	.44
July 8.....	.113	.590	4.96	.209	.103	.49
July 15.....	.102	.194	1.90	.077	.156	2.02
July 22.....	.043	.140	3.26	.042	.141	3.35
Aug. 27.....	.056	.190	3.55	.064	.144	2.25

TABLE 7.—Percentage of total and nonprotein nitrogen in oven-dried potato pulp

Date of digging	Percentage of total nitrogen—		Percentage of total nonprotein nitrogen—	
	At time of digging	At end of rest period	At time of digging	At end of rest period
June 17.....	1.73	1.67	0.400	0.459
June 24.....	1.72	1.65	.307	.503
July 8.....	1.52	1.54	.316	.494
July 15.....	1.73	1.61	.384	.481
July 22.....	1.81	1.82	.468	.651
Aug. 27.....	1.83	1.80	.590	.589

TABLE 8.—Distribution of nonprotein nitrogen in potatoes dug at different stages of development and analyzed immediately
[Data expressed in percentage of total nitrogen]

Date of digging	Total nonprotein nitrogen	Nitrogen of acid amides	Nitrogen in phosphotungstic precipitate	Mono-amino nitrogen
June 17.....	23.06	3.73	3.60	8.94
June 24.....	17.84	3.40	3.09	7.34
July 8.....	20.78	4.07	3.50	8.83
July 15.....	22.20	4.74	3.87	11.36
July 22.....	27.07	5.54	4.06	12.00
Aug. 27.....	32.18	6.58	5.49	15.05

TABLE 9.—Distribution of nonprotein nitrogen in potatoes dug at different stages of development and analyzed at the end of the rest period
[Data expressed in percentage of total nitrogen]

Date of digging	Total nonprotein nitrogen	Nitrogen of acid amides	Nitrogen in phosphotungstic precipitate	Mono-amino nitrogen
June 17.....	27.42	4.23	2.10	12.10
June 24.....	30.39	3.73	2.28	14.83
July 8.....	32.08	4.69	2.18	15.92
July 15.....	29.82	-----	2.00	14.57
July 22.....	35.58	7.24	2.11	18.07
Aug. 27.....	31.67	5.89	2.55	15.64

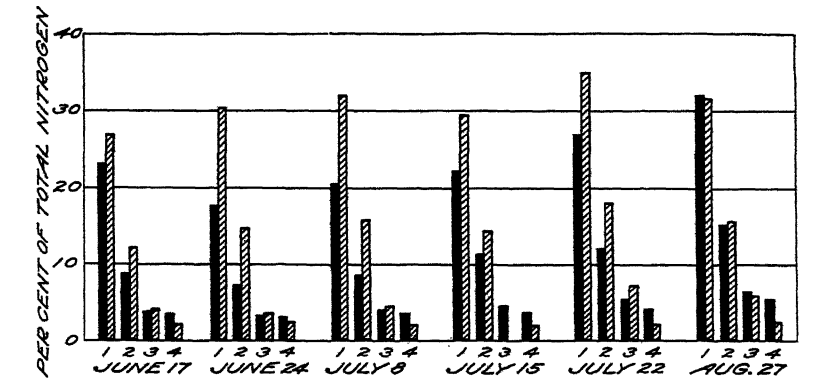


FIG. 1.—Distribution of nonprotein in potatoes at different stages of development (solid black bars) and at the end of the rest period (hatched columns). The first pair of bars of each set represents total nonprotein nitrogen, the second pair monoxamine nitrogen, the third pair amide nitrogen, and the fourth pair basic nitrogen. The dates indicate the time of digging

RESPIRATION IN MATURE AND IMMATURE POTATOES

Since respiration is the process which furnishes the energy for growth, it seemed of interest in this connection to compare the respiratory rates in mature and immature potatoes.

Experiment 1: A lot of immature Rural New Yorker potatoes was dug October 3. Another lot was allowed to mature in the ground and was dug October 30. The respiration rate at 22° C. was determined in both lots immediately after digging. The results are shown in Table 10.

TABLE 10.—*Respiration of mature and immature Rural New Yorker potatoes immediately after digging*

Condition of potatoes	Mgm. of CO ₂ per kilo per hour (average for succeeding 24-hour periods)										
	First period	Second period	Third period	Fourth period	Fifth period	Sixth period	Seventh period	Eighth period	Ninth period	Tenth period	Eleventh period
Mature.....	20.4	18.1	20.0	19.4	13.3	11.1	10.7	7.1	6.8	6.3	7.4
Immature..	33.1	23.9	20.1	15.4	13.3	20.6	18.0	13.3	11.1	9.8	8.7

Experiment 2: Samples of the same lots of potatoes used in experiment 1 were stored at 36° F. for four months. Respiration rates in the mature and immature potatoes at 22° C. were again compared. The results of this experiment are shown in Table 11.

TABLE 11.—*Respiration of mature and immature Rural New Yorker potatoes at the end of the rest period*

Condition of potatoes	Mgm. of CO ₂ per kilo per hour (average for succeeding 24 hour periods)								
	First period	Second period	Third period	Fourth period	Fifth period	Sixth period	Seventh period	Eighth period	Ninth period
Mature.....	19.3	23.0	22.1	20.7	18.8	15.6	14.1	13.0	10.8
Immature.....	18.6	22.9	23.0	21.2	18.0	15.5	13.3	11.8	10.8

Other experiments similar to the ones here recorded have all shown greater respiratory activity in the immature potatoes immediately after digging. This is probably due to the greater permeability of the tender skin to gaseous exchange. The immature potatoes were also more subject to skinning and wounding in handling. This would also increase respiration. The respiration rates in the immature and mature potatoes at the end of the rest period are of special interest in this connection. The data in Table 11 show the same respiratory activity in both lots. The higher respiration rates at the beginning are due to the fact that the potatoes were transferred from the storage temperature to the higher temperature at which the respiration determinations were made.

DISCUSSION OF RESULTS

Reducing sugars and sucrose decreased as starch increased up to the time when about 80 per cent of the leaves were dead, but the

tubers were still quite immature as judged by the skin. Sucrose predominated at all stages of development, but the ratio of sucrose to reducing sugars was highest when the tubers were making their most rapid growth. The percentage of sucrose in the immature potatoes decreased during storage so that by the end of the rest period it was practically the same in all lots. The percentages of reducing sugars which are all very low show little change during storage when calculated to basis of moisture content at time of digging.

The small percentage of starch in the very immature lot after storage is probably due to the high respiration of these tubers immediately after digging.

When calculated to a dry basis the percentages of ash, crude fiber, and total nitrogen remain fairly constant at all stages of development. The increase of nonprotein and amino nitrogen in the tubers with increasing periods on the vine show that hydrolysis or digestion of protein is one of the important ripening or maturing processes. These processes in the immature seed continued in storage so that by the end of the rest period the percentages in all lots except the one containing very immature potatoes, were practically the same and equal to those found in the mature tubers immediately after digging.

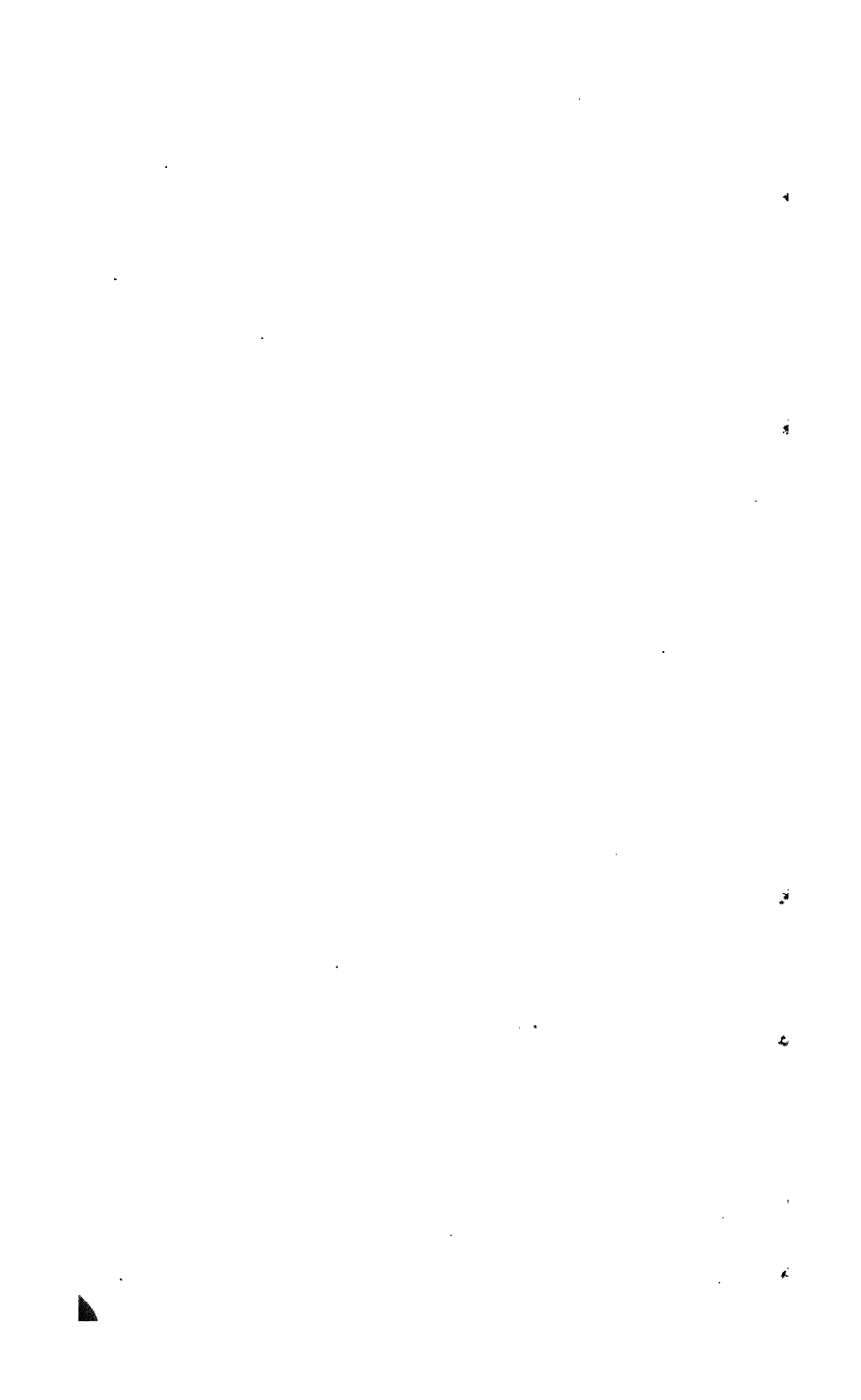
The higher amounts of nitrogen in the phosphotungstic acid precipitates of the extracts from the tubers at the time of digging may be due to proteoses and peptones which are hydrolyzed to amino acids during storage. The nitrogen of acid amides increases slightly as the tubers mature. The humin nitrogen which is formed during the hydrolysis of the acid amide nitrogen was practically the same in all lots. Since this fraction is not formed in the living tissue and is not significant in this connection the data are not recorded.

The higher respiration in immature potatoes for a period after digging appears to be due to the fact that the skins are more permeable to gases. At the end of the rest period when growth is possible, respiration is no greater in the immature than in the mature seed.

SUMMARY AND CONCLUSIONS

The ripening and maturing processes in potatoes may continue during storage, so that by the end of the rest period immature potatoes large enough for seed have practically the same percentage composition and respiratory response as potatoes allowed to mature on the vine if both are stored under the same conditions.

The data in this paper do not reveal any chemical or physiological basis for the superiority of immature potatoes for seed. The cases reported of immature seed giving better results than mature seed may have been due to greater freedom from degeneration diseases in the immature seed.



THE INFLUENCE OF THE ENVIRONMENTAL TEMPERATURE ON THE HEAT PRODUCTION OF CATTLE¹

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INTRODUCTION

The study herein reported was suggested by variations in the heat increment per unit of feed, especially as influenced by very low planes of nutrition, which were disclosed by a recent review and recalculation of the published energy metabolism work of this institute (4).²

A detail of the former experimental procedure of the institute in its respiration calorimetric work with steers, which gave especial significance to the environmental temperature in relation to the heat production of the experimental subject, was the shearing of the steer at the beginning and at the end of the season's program of experimentation to permit of an accounting for the growth of hair. Obviously this procedure would have the effect of raising the critical temperature—that is, the temperature at which the waste heat of food utilization is just sufficient to maintain the body temperature without increasing the katabolism of body substance.

Among the published experiments the one which shows most clearly the relation of environmental temperature to heat production is experiment No. 186, on the available energy of red clover hay. The results of this experiment were first published in 1908 (1), and have been discussed from time to time in later papers, with recalculation of the data in the light of progressive improvements of methods and understanding (2, 4, 6).

The present paper consists of (1) a brief review of experiment No. 186, the revised results of which have not previously been discussed in detail in relation to critical temperatures, and (2) a report of a later experiment, No. 235, conducted in 1924-25.

EXPERIMENT NO. 186

Experiment No. 186 was conducted during January, February, and March, 1904. The subject was a grade Shorthorn steer, approximately 5 years old, and the feed was red clover hay. The dates included in the experimental periods and the number of days in the calorimeter are given below.

Experimental periods	Days in calorimeter	
	At 19° C.	At 13.5° C.
1, Jan. 13-22.....	Jan. 13 and 14.....	Jan. 20 and 21.....
2, Feb. 10-19.....	Feb. 10 and 11.....	Feb. 17 and 18.....
3, Mar. 9-18.....	Mar. 9 and 10.....	Mar. 16 and 17.....

¹ Received for publication Apr. 22, 1926; issued September, 1926.

² Reference is made by number (italic) to "Literature cited," p. 589.

A summary exhibit of the results comprises Table 1.

TABLE 1.—*Experiment 186: Increments of heat production per kilogram of dry matter of feed, as affected by environmental temperature*

Period No.	Temperature of chamber	Dry matter of feed	Metabolizable energy of ration	Heat production, corrected to 12 hours' standing	Gain of energy by animal	Latent heat of water vapor, per cent of total ^a	Heat increments per kgm. of dry matter	
							Periods compared	Calories
	° C.	Kgm.	Cal.	Cal.	Cal.			
1a.....	19.0	2.933	5,922	10,597	-4,675	21.27	1a and 2a.....	321
1b.....	13.5	2.933	5,922	11,321	-5,399	14.51	1a and 3a.....	7
2a.....	19.0	5.025	10,690	11,268	-578	24.34	1b and 2b.....	-99
2b.....	13.5	5.025	10,690	11,113	-423	17.44	1b and 3b.....	-534
3a.....	19.0	4.139	8,614	10,605	-1,991	25.35	2a and 3a.....	748
3b.....	13.5	4.139	8,614	10,677	-2,063	18.85	2b and 3b.....	492

^a Uncorrected to the standard day.

In period 1 as the temperature was decreased from 19° to 13.5°, the quantity of feed remaining the same, the heat production increased from 10,597 to 11,321 Calories, the negative balance of energy increased from 4,675 to 5,399 Calories, and the latent heat of water vapor decreased from 21.27 to 14.51 per cent of the total heat production (uncorrected to the standard day). Obviously the temperature was below the critical in period 1b. That this is true is also shown by the negative heat increments, computed through comparisons involving the heat production of this period.

It is also clear that the temperature was below the critical in period 1a, as is shown by the facts that the heat increments involving period 1a are very much smaller than those derived from periods 2 and 3, and that the heat production is practically the same in period 1a as in period 3a, while the feed is materially less.

That the temperature in periods 2 and 3 was above the critical is clearly shown by the practical identity of the heat production at the two temperatures.

One condition which doubtless affected the critical temperature for this experimental subject, and at a progressively changing rate, was the growth of the hair coat. Since the animal was sheared at the beginning of the experiments (January 2), and the coat was thereafter allowed to grow, the effect of this increasing protection must have been to lower the critical temperature. The results of experiment 235 (to be discussed) give us reason to believe that the difference in the critical temperature for an animal immediately after shearing and two and one-half months afterward, as shown in experiment 186, is a factor of very great importance in determining the influence of environmental temperatures on the production of heat.

A study of the data obtained in experiment 186 makes clear the fact that neither the energy balance nor the percentage of the heat production which is represented by the latent heat of water has by itself a definite diagnostic significance in relation to the critical temperature, though the conditions prevailing in respect to both contribute to the general picture of the response of the animal to the temperature of its environment.

From the point of view of the general program of research on energy metabolism at this institute the important conclusion to be drawn

from this experiment is that even when receiving a considerable quantity of feed (2.933 kgm. of dry matter) an environmental temperature as high as 19° C. may be below the critical temperature for a steer that has been sheared. On account of the imperfect method used for computing the results, especially the heat production as observed to the standard day as to standing and lying, this point was missed in the earlier discussions of this experiment, in spite of the fact that the work was planned especially to bring out the facts in this relation.

The heat increments derived from the three periods at 19° C. were 321 Calories, 7 Calories, and 748 Calories. Except for such error as there may be in the assumption that the heat increment is a lineal function of the feed, theoretically, the only reasons (aside from uncontrolled variations) that these values should not have agreed exactly were that the temperature in periods 1a and 1b was below the critical temperature, and that the steer began the season's experiments without his coat and finished with a two and one-half months' growth of hair.

In other of the early experiments with steers at this institute there were many instances of a loss from the body of as much as 2,000 Calories a day, and in all cases the steers began the season's experimental program without their coats. It is impossible to estimate the influence which the growth of hair during the course of the experiments may have exerted on the results, but that the results were influenced by this added protection to the body of the animal must be considered as at least a possibility, and may be guarded against in the future by not shearing the experimental subjects. It has been found that the error thus introduced into the computed heat production by the failure to account for the growth of hair is properly negligible in studies of the net energy value of feeds.

EXPERIMENT NO. 235

OBJECT

The purpose of experiment 235 was to study the following problems, only the first of which is discussed in this paper: (1) The heat production of the beef steer as affected by the coat of hair and the temperature of the environment; (2) the fasting katabolism as a measure of the maintenance requirement of energy; (3) the comparative utilization of energy for maintenance and body increase; (4) a comparison of the direct and the respiratory quotient methods of measurement of heat production (*a*) during fasting, (*b*) while on a maintenance level, and (*c*) in body increase.

EXPERIMENTAL SUBJECTS

Two steers, designated Nos. 259 and 260, were used in the experiments, No. 259 being an Aberdeen Angus-Galloway cross, aged 16½ months, and No. 260, Aberdeen Angus-Shorthorn cross, aged 15¼ months at the beginning of the experiments. Both steers were of typical beef character, their sires and dams being purebred. Both were quiet and admirably adapted to experimental use. In condition they were approximately half fat. No. 259 was kept shorn throughout the experiments, while No. 260 wore his full winter

coat of hair. The steers had been halter broken and familiarized with the calorimeter and all conditions attendant upon the experimental routine before the experiments began. The live weights of the steers are given in Table 2.

TABLE 2.—Schedule of experiment No. 235

Treatment	Steer 260 (full winter coat)			Steer 259 (sheared)		
	Experimental periods	Temperature	Initial live weight	Experimental periods	Temperature	Initial live weight
		° C.	Kgm.		° C.	Kgm.
I. Fasting, total duration.	Jan. 9 to Jan. 19 ^a -----	-----	-----	Jan. 27 to Feb. 7 ^b -----	-----	-----
Calorimeter period No. 1.	Jan. 13, 6 p. m. to Jan. 14, 6 p. m.	15.54	356.8	Feb. 1, 6 p. m. to Feb. 2, 6 p. m.	13.68	349.1
Calorimeter period No. 2.	Jan. 15, 6 a. m. to Jan. 16, 6 a. m.	18.46	354.4	Feb. 3, 6 a. m. to Feb. 4, 6 a. m.	15.70	346.1
Calorimeter period No. 3.	Jan. 16, 6 p. m. to Jan. 17, 6 p. m.	21.51	352.1	Feb. 4, 6 p. m. to Feb. 5, 6 p. m.	18.26	343.1
Calorimeter period No. 4.	Jan. 18, 6 a. m. to Jan. 19, 6 a. m.	14.16	349.7	Feb. 6, 6 a. m. to Feb. 7, 6 a. m.	22.06	340.0
II. Maintenance, digestion period.	Feb. 10 to 27, inclusive.	-----	-----	Feb. 24 to Mar. 13, inclusive.	-----	-----
Calorimeter period.	Feb. 24, 25, and 26	17.73	378.7	Mar. 10, 11, and 12	17.73	357.7
III. Production, digestion period.	Mar. 10 to 27, inclusive.	-----	-----	Mar. 24 to Apr. 10, inclusive.	-----	-----
Calorimeter period.	Mar. 24, 25, and 26	17.69	411.7	Apr. 7, 8, and 9	17.76	386.1

^a Physic given at 9 and 11 a. m., on Jan. 9; last feed, grain only, given on evening of Jan. 9; paunch pumped out and enema given at 11 a. m., Jan. 10.

^b Last feed, grain only, given on evening of Jan. 27; physic given at 9 and 11 a. m., Jan. 28.

SCHEDULE OF EXPERIMENTS

In the schedule of experiments as set forth in Table 2 it will be noted that both steers were subjected to the same routine, which embraced observations on the three planes—fasting, maintenance, and production.

The fasting period was 10½ days for No. 259 and 9½ days for No. 260. After a preliminary fast of 5 days with No. 259 and 4 days with No. 260, a series of four 24-hour calorimeter experiments, separated by 12-hour intervals, each period being at a different temperature, were conducted with each steer.

The maintenance and production periods were each 18 days in length. During the first 15 days of these periods the steers were confined in digestion stalls, and the feed eaten and the excreta voided were quantitatively accounted for. The last 3 days of each 18-day digestion experiment constituted the calorimeter periods.

PREPARATION OF SUBJECTS

At the beginning of the experiments it was desired to get the steers onto a true fasting basis in the shortest possible time. The method followed with No. 260 was to give a physic and to withhold roughage for 1 day and grain for one-half day; the paunch was then washed out by means of a stomach pump and an enema given. The pump showed the paunch to be empty at this stage; hence in the preparation of No. 259 the pumping out of the paunch was omitted.

The preliminary feeding was on a maintenance basis. After the termination of the 9½ and 10½ day fasts the steers ate very little for a few days; in fact, in both cases it required 4½ days to get them back to as much as a maintenance ration.

Steer No. 259 was sheared January 27 and again March 4. The weight of the full winter coat as removed January 27 was 1,200 gm., the steer weighing at that time 373 to 384 kgm. (before and after watering). On March 4, 36 days thereafter, a second shearing yielded 52 gm. of hair. The growth of hair, therefore, seems to have been at the rate of 1.4 gm. per day.

METHODS OF EXPERIMENTATION

The methods of work conformed, in a general way, to those previously used at the institute, as detailed in various publications, and with those outlined by Forbes and Grindley (3). However, since the publication in 1918 of the last article on steer feeding such material improvements of procedure have been accomplished in the institute's respiration calorimetric studies as to place them on a new status of accuracy and promise. These changes of procedure have recently been enumerated in "Science" by one of the present writers (5).

TABLE 3.—*Experiment 235: Daily heat production of fasting steers*

Animal and temperature of chamber (° C.)	Period	Heat emission			Heat emission corrected to one-half time standing	Heat production corrected to one half time standing
		By radiation and conduction	As latent heat of water vapor	Total		
Steer No. 260 (full coat): 13.54.....	f1a.....	Cal. 2,411	Cal. 878	Cal. 3,289	Cal. 3,347	Cal. -----
	11b.....	2,122	704	2,826	2,904	-----
	Total per day.....	4,533	1,582	6,115	6,251	6,322
18.4b.....	f2a.....	2,047	997	3,044	3,126	-----
	2b.....	2,060	1,190	3,250	3,330	-----
	Total per day.....	4,107	2,187	6,294	6,456	6,430
21.51.....	f3a.....	1,839	1,391	3,230	3,315	-----
	3b.....	1,725	1,400	3,125	3,171	-----
	Total per day.....	3,564	2791	6,355	6,486	6,453
14.16.....	f4a.....	2,240	797	3,037	3,117	-----
	4b.....	2,279	746	3,025	3,114	-----
	Total per day.....	4,519	1,543	6,062	6,231	6,180
Steer No. 259 (sheared): 13.68.....	f1a.....	3,545	747	4,293	4,295	-----
	11b.....	3,304	663	3,967	4,033	-----
	Total per day.....	6,849	1,410	8,260	8,328	8,243
15.70.....	f2a.....	3,113	724	3,837	3,873	-----
	2b.....	3,178	751	3,929	3,969	-----
	Total per day.....	6,291	1,475	7,766	7,872	7,889
18.26.....	f3a.....	2,999	782	3,781	3,813	-----
	3b.....	2,967	769	3,736	3,727	-----
	Total per day.....	5,966	1,551	7,517	7,540	7,519
22.00.....	f4a.....	2,489	904	3,393	3,430	-----
	4b.....	2,460	862	3,322	3,392	-----
	Total per day.....	4,949	1,766	6,715	6,822	6,806

DISCUSSION OF RESULTS

The heat production of the fasting animals is recorded in Table 3 by 12-hour subperiods, to permit of judgment of the validity of the heat production of each day as a whole. This is rendered desirable by the fact that the one-day measurements are only one-third the length of the usual calorimeter periods. It was considered impracticable, as well as undesirable, to hold the fasting animal for the three-day period of observation at each of the temperatures.

The agreement between the 12-hour subperiods was usually excellent. With both steers the agreement of these subperiods was least perfect on the first day of the series of measurements, but even on these days it was fair. As a whole, the data are highly satisfactory in spite of the short periods of measurement, one period serving as a check on others in the series.

The heat emission was corrected to the standard day of 12 hours' standing; and then by a correction for the temperature gain or loss in water and excreta, the values for heat emission were computed to heat production. The derivation of the water balance used in this computation is indicated in Table 4.

TABLE 4.—*Experiment 235: Computation of the daily water balance in the fasting experiments with steers*

Animal and period No.	Fresh urine	Dry matter in urine		Water in urine	Fresh feces	Dry matter in feces		Water in feces	Water vapor	Total water outgo	Water drunk	Water balance
	<i>Grams</i>	<i>Per cent</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Steer 260 (full coat):												
1.....	1,210	9.15	110.7	1,099	30	34.21	10.3	20	2,690	3,809	6,960	+3,152
2.....	^b 444	8.02	35.6	408	459	24.52	112.5	347	3,725	4,480	220	-1,260
3.....	^b 688	-----	^a 41.7	647	105	31.98	33.6	71	4,775	5,493	3,590	-1,903
4.....	1,421	6.06	8.61	1,335	181	37.11	67.2	114	2,616	4,065	2,030	-2,035
Steer 259 (sheared):												
1.....	2,031	5.91	120.0	1,911	5	27.07	1.4	4	2,391	4,306	920	-3,386
2.....	^d 6	^d 5.91	.4	6	7	27.07	1.9	5	2,509	2,519	3,300	+781
3.....	2,111	^e 7.75	163.5	1,948	224	29.61	66.3	158	2,642	4,747	3,780	-987
4.....	1,785	7.75	138.3	1,647	15	30.80	4.6	10	3,024	4,681	3,770	-911

^a Assumed to be the same as in samples between periods.

^b Computed $\frac{41.7 \times 100}{6.058 \text{ (assumed)}} = 688.3$.

^c $1,832 \text{ gm. (including wash water)} \times \frac{2.274 \text{ (per cent dry matter)}}{100} = 41.7$.

^d Assumed to be the same as in period 1.

^e Assumed to be the same as in period 4.

In view of the fact that the intake and outgo of water by way of the alimentary and excretory tracts are extremely variable, little evidence of order in the water balances is expected. The outgo of water vapor, however, varies from period to period, always in the same direction as the temperature of the air in the calorimeter chamber. It will be observed that the water balances were negative in six cases of the eight recorded. This is in harmony with past observations at the institute, and perhaps signifies that the drinking facilities in the respiration chamber were not quite satisfactory to the steer. Since a steer can drink, and often does drink, at one draft, a sufficient quantity of water to meet his requirements for two or three days,³

³ The extensive water-drinking capacity of cattle is of great economic importance. In semiarid regions cattle often live month after month without drinking more than two or three times a week. This ability of cattle to go for long periods without drink makes possible the utilization for meat production of forage in regions so remote from markets and so far from water as to be useless for any other agricultural purpose.

it is readily conceivable that a very trifling factor may appreciably affect the water intake and balance.

The heat production in relation to the body surface will be discussed in connection with Table 5. Referring first to the sheared steer, No. 259, it will be noted that in the course of 4 days of observation, beginning with day of fast 5 to 6 and continuing to day 9½ to 10½, with half-day intervals between the 24-hour periods of observation, the temperature of the chamber of the calorimeter was raised by increments of 2.02°, 2.56°, and 3.80°, from 13.68° on day of fast 5 to 6 up to 22.06° on day 9½ to 10½. Thus the changes in temperature were all in one direction—that is, upward.

TABLE 5.—*Experiment 235: Computation of heat production per square meter of body surface of fasting steers*

Animal and period No.	Temperature of chamber	Days of fast	Live weight, fasting	Average live weight, maintenance periods	Empty weight, maintenance periods	Empty weight, fasting	Surface area, 0.1186W ^¾	Heat production per standard day		
								Total	Per 100 kgm. of live weight	Per square meter of body surface
Steer 259 (sheared):	° C.		Kgm.	Kgm.	Kgm.	Kgm.	Sq. m.	Cal.	Cal.	Cal.
1.....	13.68	5 - 6	339.6	350.7	315.6	304.6	4.23	8,243	2,427	1,949
2.....	15.70	6½ - 7½	336.6	350.7	315.6	301.6	4.20	7,889	2,344	1,878
3.....	18.26	8 - 9	333.6	350.7	315.6	298.6	4.18	7,519	2,254	1,799
4.....	22.06	9½ - 10½	330.5	350.7	315.6	295.6	4.15	6,806	2,059	1,640
Steer 260 (full coat):										
1.....	15.54	4 - 5	356.0	369.3	332.4	323.4	4.39	6,322	1,776	1,440
2.....	18.46	5½ - 6½	353.6	369.3	332.4	320.4	4.37	6,430	1,819	1,471
3.....	21.51	7 - 8	351.3	369.3	332.4	317.4	4.34	6,453	1,837	1,487
4.....	14.16	8½ - 9½	349.0	369.3	332.4	314.4	4.32	6,180	1,771	1,431
Average, 260 only.....									1,801	1,457

^a Moulton's formula (7).

Coincident with each increase in the temperature of the chamber, the animal showed a decrease in heat production. At a temperature of 13.68° C. the heat production was 8,243 Calories; at 15.70°, 7,889 Calories; at 18.26°, 7,519 Calories; and at 22.06°, 6,806 Calories.

Since each increase in temperature was accompanied by a large decrease in heat production, the temperature must have been below the critical for the animal in the first three periods at least; in the fourth period we have no basis for judgment as to the relation of the temperature to the critical.

With steer No. 260, which possessed his full winter coat of hair, the temperature on the four days of observation between days of fast 4 to 5 and 8½ to 9½ was raised from 15.54° to 18.46°, next to 21.51°, and then lowered to 14.16°.

During the periods of increasing temperature the heat production was essentially unchanged (6,322, 6,430, and 6,453 Calories). The slight increase noted from period to period may possibly signify an expenditure of energy in keeping cool, although the evidence on this point is inconclusive. Since these increases in temperature were not accompanied by decreases in heat production, it is obvious that the prevailing temperatures were above the critical for this steer.

After it was observed that the temperature during the first three periods seemed to be above the critical for this steer, it was lowered during the fourth period to 14.16° C. in an effort to reach a temperature below the critical. The heat production at this temperature, however, was the lowest of all. It therefore became perfectly clear that the temperature had been above the critical for this steer in at least the first three periods, and that in the fourth there was as with the other steer, no basis for judgment as to the relation of the temperature to the critical.

Thus the difference in the coat of hair of the two steers led to a sufficient difference in heat production to reveal the environment for one to be clearly above and for the other as clearly below the critical temperature—at the range of temperatures obtainable in the respiration calorimeter as now equipped. It becomes obvious, therefore, that if it is desired to relate the heat production to the feed consumption in work involving low planes of nutrition with cattle the experimental subjects must not be sheared. Fortunately, as already explained, a neglect to account for the growth of the coat does not involve us in significant error, in studies of the body metabolism as a whole.

Another observation of importance which may be made from the heat production of steer No. 260 is that the animal had practically reached the true fasting katabolism on the fourth to fifth day after the last feed.

The influence of the environmental temperature on the manner of the heat loss is shown in Table 6. With both steers the heat lost by radiation and conduction (combined) and that lost by evaporation of water varied in a complementary manner—that is, the warmer the temperature the greater the loss of heat by evaporation and the smaller the loss by radiation and conduction. This relationship prevailed invariably, both as revealed in the absolute quantities of heat and as percentages of the total heat production.

TABLE 6.—*Experiment 235: Influence of temperature on the manner of heat loss*

Animal and period No.	Temperature of chamber	Heat lost by radiation and conduction ^a	Heat lost by evaporation of water	Total heat production ^a ^b	Heat lost by radiation and conduction as per cent of total	Latent heat of water vapor as per cent of total
Steer No. 259 (sheared):	° C.	Cal.	Cal.	Cal.	Per cent	Per cent
1.....	13. 68	6, 763. 5	1, 410. 7	8, 174. 2	82. 74	17. 26
2.....	15. 70	6, 308. 7	1, 475. 0	7, 783. 7	81. 05	18. 95
3.....	18. 26	5, 944. 7	1, 550. 9	7, 495. 6	79. 31	20. 69
4.....	22. 06	4, 933. 2	1, 766. 0	6, 699. 2	73. 64	26. 36
Steer No. 260 (full coat):						
1.....	15. 54	4, 604. 2	1, 581. 4	6, 185. 6	74. 43	25. 57
2.....	18. 46	4, 081. 0	2, 186. 5	6, 267. 5	65. 11	34. 89
3.....	21. 51	3, 531. 0	2, 791. 1	6, 322. 1	55. 85	44. 15
4.....	14. 16	4, 468. 2	1, 543. 5	6, 011. 7	74. 33	25. 67

^a Corrected for gain by body.

^b Not corrected to standard day.

One pronounced difference is noticeable in the response of the two steers to the temperature changes. With about the same range of temperatures, steer No. 260 (full coat) gave off 25.57 to 44.15 per cent of the heat production as latent heat of water vapor, while steer No. 259 (shorn) eliminated 17.26 to 26.36 per cent of the heat in

this manner. This difference is due to the fact that the temperatures were below the critical for steer No. 259. Since this animal had been deprived of its coat of hair, the skin was cooler, the superficial circulation of the blood was doubtless diminished by the contraction of the capillaries, and the outgo of latent heat of water vapor was naturally lessened.

Thus far we have dealt with the heat production of the individual animal, computed to a standard day as to standing and lying, but no effort has been made to express the heat in relation to a unit of size of the animal. Two practicable units of reference are those representing live weight and surface area. Each has its point of superiority. Thus, live weight can be ascertained with relative accuracy, and without extensive compromise as to significance on account of variation in the content of the alimentary and excretory system, but it has the disadvantage of not varying directly as the heat production. Surface area, on the other hand, has the advantage of varying more nearly as does the heat production; but in using this measure one is handicapped by the fact that there is no perfect means for determining the surface area of the animal.

Among the possible means for determining the surface area are (1) computation in accord with the two-thirds power of the live weight; (2) direct measurement on the living animal, as by Brody's "surface integrator"; and (3) reference to formulae, based either upon Brody's surface measurements or upon measurements of the freshly removed skins of animals of the kind used in the experiment. There are obvious possibilities of error in all of these methods. In this study the heat production is expressed (1) per head, (2) per kilogram of live weight, and (3) per square meter of body surface, the surface area being computed as in Table 5.

In this computation, beginning with the average live weights as observed in maintenance periods, the empty live weights in maintenance periods were computed by the use of the factor 0.9, taken from the work of Trowbridge, Moulton, and Haigh (8). Next, from these empty live weights during maintenance were computed the empty live weights during fast, by subtracting from the former values the estimated loss of body tissue during fast. In this computation the protein loss was computed from the urinary nitrogen, and the carbon balance was computed to fat, the carbon lost as carbon dioxide plus the carbon in the urine being considered as the total loss of carbon derived from body tissue.

From the empty live weights during fast the surface areas during fast were computed by the use of Moulton's formula $0.1186W^{.667}$ (7). The most obvious possibility of error in this procedure seems to be in the measurements of the hides, as indicating the surface area of the animals, the uncontrolled factors being the folding of the skin and its tension. Not only does the skin lie to some extent in folds on the live animal, but obviously a difference in tension must accommodate the difference in surface area caused by the "fill" of the animal.

The heat production on the three above-mentioned bases is presented in the three columns at the right in Table 5. Average values for steer No. 259 are not presented, since these would be without definite significance in relation to a normally covered animal, but for steer No. 260 averages of 18.01 Calories per kilogram of live weight and 1,457 Calories per square meter of body surface are presented as

applying to normal cattle. The latter datum is of especial significance and value in the computation of the maintenance requirement of energy.

The decrease in heat production per square meter of body surface per degree of rise in temperature, as observed with steer No. 259, and derived as indicated in Table 7, is a figure of fundamental significance, especially as indicating the rate of loss of heat, or saving of heat (according to circumstances), as affected by temperatures below the critical. On the basis of the evidence derived from this experiment it appears that a rise of 1 degree in temperature below the critical occasions a saving of 36 Calories per square meter of body surface.

TABLE 7.—*Experiment 235: Effect of subcritical temperatures on the daily heat production of steer No. 259*

Periods compared	Rise in temperature of chamber	Decrease in heat production per square meter of body surface	Decrease in heat production per square meter of body surface per degree of rise in temperature
	° C.	Calories	Calories
1 and 2.....	2.02	71	35
1 and 3.....	4.58	150	33
1 and 4.....	8.38	309	37
2 and 3.....	2.56	79	31
2 and 4.....	6.36	238	37
3 and 4.....	3.80	159	42
Average.....			36

The practical significance of this fact, which is so clear as to require no special demonstration, is that under the usual conditions of intensive cattle feeding, where feed and shelter are to be had at reasonable cost, it is more profitable to keep the animal above the critical temperature than to force it to burn extra feed or to draw upon body reserves of energy in order to keep warm. Whatever the conditions as to profitable practice, however, it is important to know that a fall of 1° C. in temperature below the critical increases the cost of maintenance of cattle by about 2.5 per cent.

Use may also be made of the value for decrease in heat production per square meter of body surface per degree of rise in temperature below the critical in computing the critical temperature of an animal. In order to do this it is necessary to have the heat production at two points, one above and one distinctly below the critical temperature. By such procedure it is possible to study the effect of various factors of treatment on the critical temperature. The data in Table 7 were obtained with an approximately half-fat steer weighing 335 kgm. No information is available to show the accuracy with which the result determined would apply to animals of other weights and degrees of fatness.

CONCLUSIONS

A recent review and recalculation of published energy metabolism work of this institute has brought to light the fact that in one experiment at least, and perhaps in others, there was a confusion of heat produced from the feed with heat produced from the katabolism of body tissue, by reason of the fact that the shearing of the steer which served as the experimental subject had the effect of raising the critical temperature for the animal above the temperature of the chamber of the respiration calorimeter.

A steer receiving as much as 2.933 kgm. of dry matter of feed per day may find a temperature of 19° C. below its critical temperature if the animal has been sheared.

In a fasting experiment, with a steer, beginning four days after the last feed was given, the heat production on the first day of observation—that is, on day 4 to 5 after feeding—was found to represent essentially the true fast.

In the case of a fasting steer, shorn of its coat of hair, weighing 339.6 to 330.5 kgm., at environmental temperatures of 13.68°, 15.70°, 18.26°, and 22.06° C., the 24-hour heat production was, respectively, 8,243 Calories, 7,989 Calories, 7,519 Calories, and 6,806 Calories, showing that at least the first three temperatures were below the critical for this animal.

With a fasting steer carrying a full coat of hair, weighing 356 to 349 kgm., and exposed to environmental temperatures of 15.54°, 18.46°, 21.51°, and 14.16° C., the corresponding 24-hour heat production was 6,322 Calories, 6,430 Calories, 6,453 Calories, and 6,180 Calories, respectively, at least the first three temperatures being above the critical. The data suggest that at these temperatures there may have been a slight expenditure of energy by the animal in keeping cool.

With both the shorn steer and the steer with a full coat the percentage of the heat emission as latent heat of water vapor increased with the increase in temperature, this effect being much more pronounced with the steer having the full coat of hair. Conversely, the percentage of the heat emission lost by radiation and conduction (combined) decreased with increase in the temperature.

The average heat production of a fasting steer with its full winter coat was 18.01 Calories per kgm. of live weight and 1,457 Calories per square meter of body surface.

The decrease of heat production per degree centigrade of rise in temperature below the critical was 36 Calories per square meter of body surface, or about 2.5 per cent of the fasting katabolism.

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THE FASTING KATABOLISM OF DRY COWS¹

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INTRODUCTION

This paper presents results on one aspect of a general investigation of the energy metabolism of milk production. The research as a whole was planned by the late H. P. Armsby, former director of the Institute of Animal Nutrition, Pennsylvania State College, the experiments here discussed having been conducted in the years 1919 to 1922, under the immediate direction of J. August Fries, with the cooperation of W. W. Braman, D. C. Cochrane, Max Kriss, C. D. Jeffries, W. J. Sweeney, and R. M. Meredith. The reader is referred to other papers (4, 1, 2, 3)² which have been published on this project.

The fasting katabolism of dry cows is of special interest in connection with the maintenance requirement in lactation, and this paper may be regarded as introductory to a paper (3) which is to follow, which is based upon studies with the same individual animals in experiments on the production of milk.

In utilizing the directly determined fasting katabolism as a measure of the maintenance requirement of energy the writers are departing from the previous method of the institute, which, following Armsby, was to compute the fasting katabolism (maintenance requirement) from the difference in heat production resulting from a corresponding difference in feed at two planes of feeding. The evidence which seems to the writers to justify this change, and a discussion of the whole subject of determination of the maintenance requirement of energy, will be presented at an early date. Suffice it to say, for the present, that the new procedure referred to simply involves the use of a directly determined instead of a computed measure, without change in theory, and that the new procedure brings this institute into harmony, in this regard, with the students of the energy metabolism of animals generally. However, the use of the writers' new measure of the maintenance requirement for cattle involves most important consequences in the determination of net-energy values.

It will readily be appreciated that in making use of the fasting katabolism as a routine measure it is necessary that it be determined in a standard way. The evidence necessary for the establishment of such a standard procedure is not yet at hand, but will be at an early date; and the present paper is a contribution toward this object.

A question which naturally suggests itself to anyone who is interested in the fasting katabolism in connection with the practical problems of animal feeding is as to the real significance of this measure

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² Reference is made by number (italic) to "Literature cited," p. 595.

in such relations. Is the heat production of a starving animal wholly the result of normal processes, or is the starving animal's metabolism essentially abnormal and deranged, in such sense that it can not be regarded as the true maintenance quota of net energy during feeding?

The metabolism of fasting is characterized by several conditions, which, whether they be regarded as normal or abnormal, do differ distinctly from those prevailing during feeding. Thus, during fast there is, of course, a depletion of not only the body content of carbohydrate and fat, but of all essential nutrients, including proteins, mineral salts, and vitamins; also there is commonly a negative water balance, at least a mild degree of acidosis, and Lennox (6) now reports a retention of uric acid.

Furthermore, rations for cattle always contain a much larger proportion of carbohydrate in comparison with fat and protein than that present in the body nutrients katabolized by fasting cattle; and, since each of these classes of nutrients is utilized at its own individual rate of efficiency, it follows that the heat production of fasting must stand in a quantitative relation to the nutrients consumed different from that prevailing during the use of any normal ration.

As for the nature of the influence, upon the associated heat production, of the several conditions of metabolism which are characteristic of fasting, the existing knowledge is far from complete but is sufficient perhaps to justify the discussion of the conception of a hypothetical "normal" maintenance requirement of energy differing in amount from the observed fasting katabolism which the writers are adopting as their measure of the maintenance quota.

The writers have found, in experiments the results of which are as yet mostly unpublished, that when they compute the fasting katabolism (as a measure of the maintenance requirement) from the difference in heat production at two supermaintenance levels of feed intake, they obtain a value which is significantly lower than the directly determined fasting katabolism. This shows that supermaintenance heat increments are larger than submaintenance increments, or, in other words, that the heat increment is not a straight-line function of the amount of the feed, and, consequently, that it is improper thus to use supermaintenance increments to apply to submaintenance conditions. Whether the hypothetical "normal" maintenance requirement of energy would be the same as or higher or lower than the fasting katabolism (maintenance requirement) as computed from supermaintenance heat increments is, in the light of the present limited knowledge, a debatable question; that is, it is not known whether supermaintenance heat increments are higher than submaintenance increments on account of heat-stimulating effects of "abnormal" or other characteristic conditions of fasting, or on account of actual differences in utilization of feed energy, because the shape of the curve of heat increments in relation to feed consumed, from a state of fast up to maximum feed consumption, is not known.

However, from the point of view of the writers' present interest in the significance of the fasting katabolism as related to metabolism during feeding, they dismiss the whole subject of the origin, composition, and essential nature of the fasting katabolism (as to its being "normal" or "abnormal"), because, whatever the facts in this situation, the amount of food which is capable of entirely preventing this katabolism of body substance (during fast) must be

credited with a net-energy value measured by the protection thus afforded the body from loss; in other words, the heat production of fasting, which represents the loss of energy by the body, is the measure of the net-energy value of a ration required to keep the animal in energy equilibrium.

In this light the fasting katabolism may be regarded as of direct and unqualified significance as a measure of the maintenance requirement of energy, in relation to feeding practice.

EXPERIMENTAL DATA

Referring to Table 1, and proceeding from the left to the right in discussing the data, the first column signifies that in experiments 221D, 221E, and 221F, cows 886, 885, 874, and 887 were subjected to respiration calorimetric studies, two on the second and third days of fast, one on the fifth and sixth days of fast, and two on days 6½ to 7½ and 7½ to 8½.

TABLE 1.—Energy metabolism of fasting dry cows, computed to calories per square meter of body surface

Experiment; animal; period; and day of fast	Live weight, fasting	Live weight, maintenance periods	Empty live weight, maintenance periods	Empty live weight, fasting (W)	Body surface (0.1186 × W ²)	Heat production per day
1	2	3	4	5	6	7
221D, 886, IV:	<i>Kgm.</i>	<i>Kgm.</i>	<i>Kgm.</i>	<i>Kgm.</i>	<i>Sq. m.</i>	<i>Cals.</i>
Day 2-3	411	420.2	378.2	376.2	4.83	6,752
Day 3-4	405			374.2	4.81	6,336
221D, 885, IV:						
Day 2-3	420	426.4	383.8	381.8	4.87	6,760
Day 3-4	417			379.8	4.86	6,570
221E, 885, III:						
Day 5-6	421	434.4	391.0	383.0	4.88	6,601
Day 6-7	419			381.0	4.87	6,376
221F, 874, III:						
Day 6½-7½	395	415.6	374.0	361.0	4.70	6,996
Day 7½-8½	393			359.0	4.69	6,658
221F, 887, III:						
Day 6½-7½	301	320.5	288.5	275.5	3.97	6,019
Day 7½-8½	297			273.5	3.96	6,228

Experiment; animal; period; and day of fast	Heat production, standard day	Heat production, per square meter of body surface	Heat production, per kilogram of body weight	Latent heat of water vapor	Temperature of chamber
	8	9	10	11	12
221D, 886, IV:	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>	<i>° C.</i>
Day 2-3	6,779	1,404	16.49	20.72	17.54
Day 3-4	6,400	1,331	15.80	20.72	17.54
221D, 885, IV:					
Day 2-3	6,523	1,339	15.53	26.38	17.63
Day 3-4	6,374	1,312	15.29	25.31	17.59
221E, 885, III:					
Day 5-6	6,384	1,308	15.16	22.39	18.41
Day 6-7	6,413	1,317	15.31	22.14	18.45
221F, 874, III:					
Day 6½-7½	6,906	1,469	17.48	21.47	17.94
Day 7½-8½	6,669	1,422	16.97	22.48	17.98
221F, 887, III:					
Day 6½-7½	5,778	1,455	19.20	19.72	17.92
Day 7½-8½	5,982	1,511	20.14	18.77	17.90

In all cases the animals were on practically a maintenance basis of nutrition when the fasting experiments began. In all cases the rations were composed of 40 per cent of alfalfa hay and 60 per cent of a grain mixture consisting of 30 per cent corn meal, 30 per cent wheat bran, 30 per cent ground oats, and 10 per cent linseed meal. The kilograms of dry matter of this ration fed to each of the cows during the preliminary treatment are the following: Cow 886, 3.782; cow 885 (experiment 221D), 3.851; cow 885 (experiment 221E), 3.448; cow 874, 4.003; and cow 887, 3.627.

In view of the differences in the length of these fasting experiments, from 3 to 8½ days, it is obvious that the results are not perfectly comparable. However, the agreement of the results, shows that in spite of the differences in length of fasts, the heat production was in no case very different from that in any other.

The live weights as observed in the fasting periods comprise the second column of Table 1, but inasmuch as the heat production varies more nearly with the surface area than with the live weight it is necessary to compute the body surface for each cow in each period. This computation is outlined in the next four columns of figures in Table 1. The third column gives the observed live weights in the maintenance periods. The fourth column gives the empty live weights in the maintenance periods, these weights having been computed from the live weights, as observed, by the use of the factor 0.9 taken from the work of Trowbridge, Moulton, and Haigh (8). The empty live weights, fasting, (fifth column) were computed by subtracting from the weights in the fourth column the computed loss of tissue during fast. The body surface (sixth column) was computed from the figures in the fourth column by the use of Moulton's formula, $0.1186 \times W^{5/8}$ (7). The writers are fully conscious of the considerable measure of assumption involved in this computation of the surface area of their experimental subjects, but are unable to do better at this time.

The seventh, eighth, ninth, and tenth columns set forth the heat production, on various bases. The seventh column is the heat production as observed, by direct calorimetry. In the eighth column this heat production is computed to the standard day, as to standing and lying, by the method of Fries and Kriss (5). The ninth column is the heat production per square meter of body surface. In the tenth column the heat production is related to the live weight.

In the eleventh and twelfth columns are set forth the latent heat of water vapor as per cent of the total heat production, and the temperature of the chamber of the calorimeter, these data being recorded on account of the possibility of their being of interest in relation to the critical temperature.

The most significant data presented here are those in the ninth column, representing the heat production in Calories per square meter of body surface. In studying these figures it is necessary to keep in mind the fact that in four of the five fasting periods the subjects were different individuals, and that the two fasting experiments with cow 885 were separated by an interval of about a year; also it should be understood that the data for heat production cover not only the basal metabolism, but, in addition, the normal activity of the cows, as when confined to a stall.

With both cows 886 and 885 the heat production on the third day of fast was less than on the second; and with cow 885 the heat production on the third day was essentially the same as on the fifth and sixth days.

With cows 874 and 887 the heat production on days $6\frac{1}{2}$ to $7\frac{1}{2}$ and $7\frac{1}{2}$ to $8\frac{1}{2}$ of fast was higher than that of cows 886 and 885 at any time. Presumably this higher production was due not to the greater duration of the fast but to individuality.

While these results are obviously promising, the writers refrain from drawing conclusions until they can be considered together with later data of similar character which will soon be available.

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TOXICITY OF DIPYRIDYLS AND CERTAIN OTHER ORGANIC COMPOUNDS AS CONTACT INSECTICIDES¹

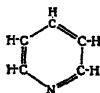
By CHARLES H. RICHARDSON, *Entomologist, Fruit-Insect Investigations, Bureau of Entomology*, and C. R. SMITH, *Associate Chemist, Insecticide and Fungicide Laboratory, Miscellaneous Division, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

Although the structural formula of nicotine has long been established, there is little prospect of an early accomplishment of the technical synthesis of this compound. However, compounds related to nicotine in structure may possess valuable insecticidal properties, so it was decided to prepare and test a number of these related compounds to determine their value as insecticides. As pyridine forms an essential part of the nicotine molecule it was selected as a starting point. A previous publication discussed the toxicity of some pyridine derivatives (3)² and referred briefly to the compounds which form the subject of the present investigation. It was found in these experiments that impure $\gamma\gamma$ dipyridyl was much more toxic than the purified substance, and it was later shown that these impurities consisted principally of isomeric dipyridyls. It is the purpose of this paper to report on the toxic value of these dipyridyls as contact insecticides. In addition, the toxicities of some other organic compounds used during the course of this work will be discussed.

THE CHEMICAL RELATIONSHIPS OF THE DIPYRIDYLS

In order to understand the chemical structure and properties of the dipyridyls, it is necessary first to consider pyridine, the compound from which they are derived. A number of drugs, including coniine, atropine, and cocaine, are derivatives of pyridine, and as a constituent of the nicotine molecule pyridine has more than the usual interest to the entomologist. Essentially, it is benzene with one of the CH groups replaced by nitrogen, and may be represented by the following graphic formula:



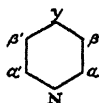
Pyridine differs from benzene in being distinctly basic and highly soluble in water, but resembles benzene in its great stability and in the unsaturated condition of its carbon atoms. Its toxic action as a contact insecticide seems to be low (3).

Under appropriate conditions, pyridine reacts with certain compounds to form substitution products in which the substituted atom or group displaces a hydrogen atom from pyridine and becomes

¹ Received for publication March 11, 1926; issued October, 1926.

² Reference is made by number (italic) to "Literature cited," p. 609.

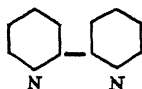
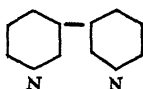
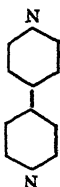
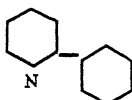
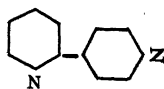
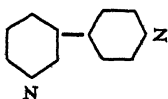
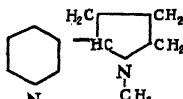
attached to the pyridine ring. There are five carbon atoms in the pyridine ring which may be involved in this way; and their positions are generally designated as alpha (α), beta (β), gamma (γ), alpha' (α'), and beta' (β') according to the following scheme:



The dipyrindyls are derivatives of pyridine composed of two pyridine molecules linked together with the elimination of one atom of hydrogen from each molecule. When metallic sodium reacts with pyridine at room temperature, a blue compound, sodium dipyrindyl, is formed (4). Sodium dipyrindyl (C_5H_5N)₂Na has two pyridine molecules linked together and one sodium atom attached to the nitrogen atom of one of the pyridine rings.

Oxidation of sodium dipyrindyl with moist air results in the formation of $\gamma\gamma$ dipyrindyl, the sodium atom and two hydrogen atoms being removed. Careful regulation of the temperature during the preliminary sodium-pyridine digestion, followed by oxidation (with dry air or oxygen) of the sodium dipyrindyls thus formed, leads to the production of an oil which contains chiefly $\alpha\alpha$, $\beta\beta$, $\beta\gamma$, and $\gamma\gamma$ dipyrindyls. This crude dipyrindyl oil, from which most of the $\gamma\gamma$ dipyrindyl was removed, was used in the experiments described below. The other dipyrindyls, the $\alpha\beta$ and $\alpha\gamma$ compounds, are also known and have been detected in the crude oil.

This completes the list of possible isomers in this series of compounds, $\beta\gamma$ dipyrindyl being discovered and made available for the first time as a result of the chemical studies of these compounds (4). They may be represented by the following formulae; the formula of nicotine is added for comparison.

 $\alpha\alpha$ dipyrindyl $\beta\beta$ dipyrindyl $\gamma\gamma$ dipyrindyl $\alpha\beta$ dipyrindyl $\alpha\gamma$ dipyrindyl $\beta\gamma$ dipyrindyl

Nicotine

The constants and properties of the isomeric dipyridyls are given in Table I (4):

TABLE I.—Constants and properties of the isomeric dipyridyls

Compound	Melting point	Boiling point	Melting point of picrate	Solubility in water
	° C.	° C.	° C.	
$\alpha\alpha$ dipyridyl.....	69.5	272.5	155	Only slightly soluble.
$\alpha\beta$ dipyridyl.....	Liquid.	287-289	148.5	Insoluble.
$\beta\beta$ dipyridyl.....	68	296	232	Soluble in all proportions.
$\gamma\gamma$ dipyridyl.....	114	305	213	Soluble in hot water.
$\beta\gamma$ dipyridyl.....	61	287	215	Very soluble in cold water, slightly soluble in hot water.
$\alpha\gamma$ dipyridyl.....	Liquid.	280-282	208	Insoluble.

The dipyridyls are solids or liquids at the ordinary temperatures. They have relatively high boiling points, and exhibit marked differences in their solubilities in water.

These compounds are basic but much weaker in this respect than pyridine. The $\alpha\alpha$ and $\beta\beta$ dipyridyls are slightly volatile when a current of steam is passed through their solutions, but the other isomers obtained in this investigation are practically nonvolatile under these conditions.

There are certain similarities between nicotine and $\beta\beta$ dipyridyl that should be emphasized at this point. Indeed, the similarity between this series of compounds and nicotine is sufficiently marked to have led chemists at one time to consider nicotine as a derivative of a dipyridyl (1). Nicotine is a β pyridyl- n -methylpyrrolidine; it consists therefore of a pyridine ring with a molecule of normal methylpyrrolidine attached at its β position. It resembles $\beta\beta$ dipyridyl further in its solubility in water, but is a much stronger base, has a considerably lower boiling point (247° C.), and is much more volatile in steam. $\beta\gamma$ dipyridyl also resembles nicotine in regard to the linkage through the β position of a pyridine ring and in its solubility relationships.

PRELIMINARY EXPERIMENTS ON THE TOXICITY OF CRUDE DIPYRIDYL OIL

Studies on the toxicity of the dipyridyls as contact insecticides were begun in August, 1920. The first indication of toxic action appeared when a crude mixture, the result of an attempt to produce $\gamma\gamma$ dipyridyl, was found to be highly toxic to the bean aphid (*Aphis rumicis* L.) at a concentration of 1 per cent of the mixture.³ Experiments soon demonstrated that purified $\gamma\gamma$ dipyridyl was only slightly toxic, if at all, and that the mother liquor separated from it was even more poisonous to insects than the original mixture. These experiments were made with samples obtained from pure pyridine which had a boiling point of 115° C. An oily mixture resulting from the action of sodium upon the homologs of pyridine (boiling point from 140° to 150° C.) yielded an ether-soluble and water-soluble fraction, each of which was considerably less toxic than the crude oil from pyridine. An oil similarly derived from crude alpha picoline

³ All percentages which refer to concentrations of solutions or mixtures are to be understood as grams dissolved or emulsified in 100 c. c. of the solution or emulsion, respectively.

also proved to be less poisonous than that from pyridine. From these experiments it seemed probable that a highly toxic compound or group of compounds existed in the oil derived from pyridine, but that the compounds derived from the higher pyridine bases by this reaction were much less toxic.

METHODS OF APPLICATION OF CRUDE DIPYRIDYL OIL

After a large number of preliminary experiments, a crude dipyridyl oil known to contain a variable mixture of $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyridyls was prepared for the toxicity experiments. It was dark brown in color and contained a small quantity of an insoluble tarry substance, besides traces of other dipyridyls and unchanged pyridine. It was partially soluble in water, and had a specific gravity of about 1.12 at 30° C.

Mixtures⁴ were prepared to contain a known weight of the dipyridyl oil in a volume of 100 c. c. Usually 0.3 per cent (moisture-free basis) of sodium-base fish-oil soap, prepared according to Quaintance and Siegler (2), was added to increase the wetting and spreading of the solution.

The solutions were applied to the insects in two ways—by spraying and by submergence of the insects in them. In the spraying experiments the solutions of crude dipyridyl oil were applied with a small hand atomizer, special care being taken to cover all surfaces of the plant with the liquid. To obtain the approximate toxic concentration, it was sufficient to spray potted plants or portions of plants infested with aphids with a graded series of concentrations; the plants were kept fresh in a bottle which contained water. For more accurate results, a portion of an infested plant was placed in a bottle containing water, the opening was closed with cotton, and the bottle placed on a piece of paper. A barrier of adhesive material about the edges of the paper prevented the escape of the aphids. Eighteen or 24 hours after spraying the insects were counted and the percentage of dead determined.

A somewhat different procedure was followed in the submergence experiments. In these the insects were placed in a small dish, the solution to be tested was poured over them, and the dish was rotated gently for 1 minute, the purpose of this movement of the dish being to keep the insects in intimate contact with the solution. Then the solution was quickly decanted, and the insects were placed on dry filter paper to remove the surplus liquid. Afterwards they were placed in large beakers covered with muslin and supplied with food. The percentage of dead insects was determined within 18 to 24 hours after submergence.

INSECTS USED IN THE EXPERIMENTS

Six species of aphids, the adults of 1 species and the larvae of 2 species of Coleoptera, and the larvae of 2 species of Lepidoptera, were used in these experiments, as follows: The bean aphid (*Aphis rumicis* L.) on dwarf nasturtium (*Tropaeolum majus*), the spinach aphid (*Myzus persicae* Sulz.) on cabbage, the pea aphid (*Illinoia pisi* Kalt.) on garden peas, the turnip aphid (*Rhopalosiphum pseudo-brassicae* Davis) on turnips, the rosy aphid (*Anuraphis roseus* Baker) on apple, the apple aphid (*Aphis pomi* De Geer) on apple; and larvae

⁴ All mixtures of crude dipyridyl oil and soap contained the oil partly in solution and partly emulsified

and adults of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), larvae of the three-lined potato beetle (*Lema trilineata* Oliv.), larvae of the Mediterranean flour moth (*Ephestia kuehniella* Zell.), and larvae of the silkworm (*Bombyx mori* L.).

SPRAYING EXPERIMENTS WITH APHIDS

Nasturtium plants infested with *Aphis rumicis* were sprayed with crude dipyriddyil oil and nicotine. Figure 1 illustrates the comparative toxicity of the two compounds to this insect.

The points in Figure 1 represent actual experiments; the curves follow the mean values.

The curve for dipyriddyil oil is derived from the following data:

Per cent concentration	Number of experiments	Number of aphids
0.02-----	10-----	1,380
.03-----	4-----	542
.04-----	10-----	1,459
.05-----	4-----	350
.06-----	4-----	396
.08-----	1-----	111
.10-----	1-----	105
.11-----	1-----	128

The curve for nicotine is based on the following:

Per cent concentration	Number of experiments	Number of aphids
0.005-----	2-----	296
.006-----	2-----	420
.007-----	2-----	329
.009-----	2-----	317

The asterisks in Figure 1 represent experiments in which the number of aphids killed was estimated. When the toxicity is high it is possible to make very close estimates of the percentage killed. Of course, when all the insects are killed the estimate is as exact as an actual count.

0.3 per cent sodium-base fish-oil soap was added to all the solutions used in these experiments.

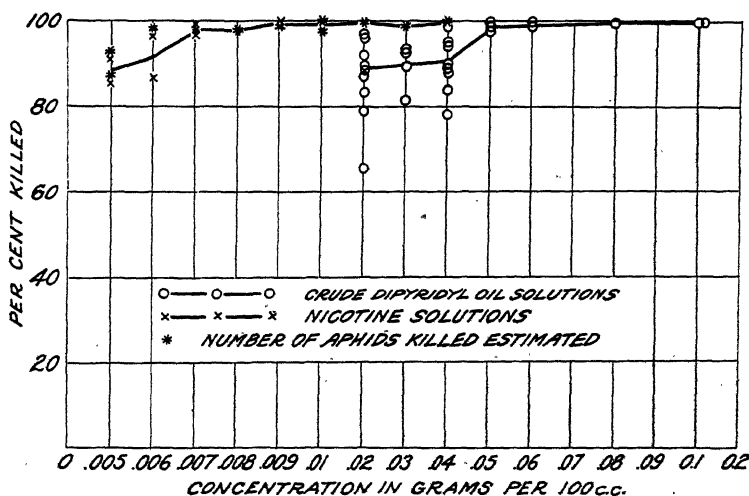


FIG. 1.—Comparison of the toxicity of crude dipyriddyil oil and nicotine to *Aphis rumicis* on nasturtium plant (spraying experiments)

Crude dipyrldyl oil killed more than 95 per cent of the aphids on nasturtium plants, at approximately 0.05 per cent concentration; and nicotine was equally toxic at about 0.007 per cent concentration. Tattersfield and Morris in a recent paper (5) give toxicity figures for nicotine which indicate an equivalent toxicity to *Aphis rumicis* with concentrations of 0.08 to 0.1 per cent, or about 12 to 14 times higher than the value given above. Aside from differences in the methods of application and the spreader used, those workers employed only the adult apterous agamic females in their experiments. All the work on *A. rumicis* mentioned above was done on adults and young of various ages as they occurred naturally on the nasturtium plants.

A number of spraying experiments were made on *Myzus persicae* which infested young cabbage plants. Crude dipyrldyl oil at a concentration of 0.05 per cent gave 100, 98.4, and 95+ per cent mortality, respectively, in three trials, but at a concentration of 0.01 per cent many living aphids remained on the plants. The concentration necessary to kill 95 per cent or more of these aphids would therefore appear to lie close to 0.05 per cent. In a preliminary series of experiments the toxic concentration for nicotine lay between 0.007 and 0.01 per cent. Unless otherwise stated, 0.3 per cent sodium fish-oil soap was used in all solutions and mixtures. Its toxicity to *Aphis rumicis* has previously been given (3, p. 8).

Six spraying tests with crude dipyrldyl oil involving 883 individuals of *Illinoia pisi* were made with the following results: In two tests at 0.05 per cent concentration, 86.5 and 91.7 per cent mortality; in two tests at 0.1 per cent, 97 and 100 per cent mortality; and in two tests at 0.69 per cent, 100 per cent mortality. Soap was added to these solutions as indicated above.

Turnip plants infested with 200 to 800 individuals of *Rhopalosiphum pseudobrassicae* were sprayed with crude dipyrldyl oil and nicotine. In the nine tests the dilutions ranged from 0.05 to 1.1 per cent; the fish-oil soap concentrations were 0.1 and 0.3 per cent. Twenty-five one-hundredths per cent of the dipyrldyl oil killed 95 per cent or more of the aphids. Under similar conditions nicotine required a concentration of about 0.05 per cent (seven experiments).

A series of nine experiments was made with the rosy aphid (*Anuraphis roseus* Baker) on apple twigs. The soap concentrations used were 0.6, 0.3, and 0.1 per cent. Since this aphid showed a rather high susceptibility to fish-oil soap solutions, 0.1 per cent soap was employed in most of the sprays. The concentrations of dipyrldyl oil ranged from 0.05 to 0.2 per cent, the latter giving approximately a 95 per cent mortality. Two comparative tests were made with a commercial nicotine sulphate solution containing 0.05 per cent nicotine and 0.3 per cent fish-oil soap. In these tests 87 and 100 per cent of the aphids were killed. This dosage is close to the field recommendation of $\frac{3}{4}$ pint of nicotine sulphate containing 40 per cent nicotine in 100 gallons of water (i. e., 0.0375 per cent nicotine).

Portions of a small Siberian crab apple tree infested with *Aphis pomi* De Geer were sprayed with 0.2 per cent crude dipyrldyl oil mixture containing 0.1 or 0.6 per cent fish-oil soap. One hundred per cent of the aphids were killed in the two tests. No experiments with nicotine were made; the field recommendation of nicotine

sulphate generally given for this aphid, however, is equivalent to 0.0375 per cent nicotine in a solution containing from about 0.3 to 0.5 per cent of moisture-free soap.

SUBMERGENCE TESTS WITH OTHER INSECTS

Larvae of the Colorado potato beetle were submerged in solutions of crude dipyrldyl oil and nicotine, with results that are shown graphically in Figure 2. The submergence time in these experiments was one minute.

Each point on Figure 2 is the result of a single experiment involving 25 larvae. The curves follow the mean values. Fifty larvae were used in the tests with 3.4 per cent nicotine, and 75 larvae with 1.1 per cent dipyrldyl oil. All other concentrations involved

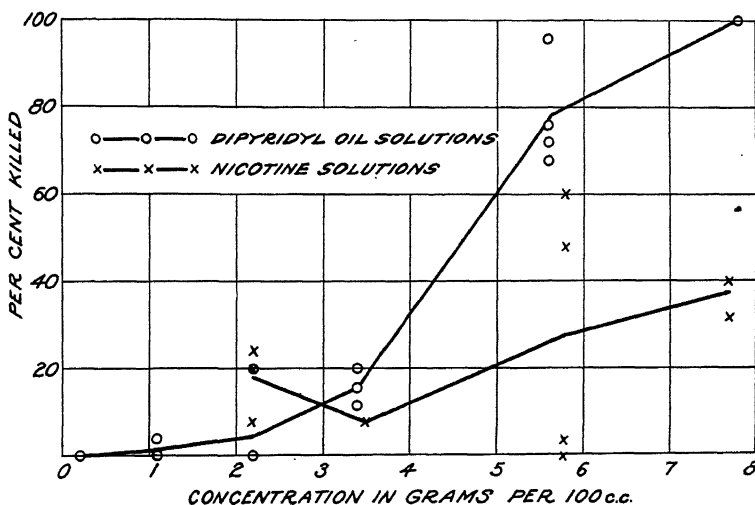


FIG. 2.—Comparison of the toxicity of crude dipyrldyl oil and nicotine to larvae of *Leptinotarsa decemlineata* (submergence experiments)

100 larvae each. The weight of the larvae ranged from 2.6 to 4.9 gm. per lot of 25 individuals.

All solutions contained 0.3 per cent of sodium fish-oil soap. Five lots of 25 larvae each submerged in 0.3 per cent soap solution for one minute were unharmed.

At all concentrations, except possibly the lowest, dipyrldyl oil was more toxic than nicotine. The solutions of nicotine which contained 3.4 per cent or more of nicotine paralyzed the larvae to such an extent that they always failed to recover, although in some cases they were alive 48 hours after treatment.

A few tests with adults of the Colorado potato beetle indicate that this stage of the insect is more susceptible to submergence in nicotine solutions than in crude dipyrldyl oil mixtures. Two tests with 1 and 1.1 per cent nicotine solutions gave 100 per cent mortality (29 beetles). Three tests with 2, 2.5, and 2.6 per cent dipyrldyl oil mixtures gave 100, 100, and 93.3 per cent mortality, respectively (37 beetles), whereas tests with 0.97 and 1.3 per cent solutions of dipyrldyl oil

gave 80 and 28.6 per cent mortality, respectively (26 beetles). The 0.3 per cent soap concentration used in these experiments resulted in a mortality of 9.2 per cent (65 beetles). These experiments, while probably inadequate to establish the approximate toxicity, appear to indicate a higher value for nicotine than for dipyrindyl oil.

When larvae of *Lema trilineata* were submerged in solutions of crude dipyrindyl oil and nicotine under comparable conditions rather striking differences were observed, as Figure 3 shows.

The submergence time in these experiments was 1 minute. Each point in Figure 3 represents an experiment; the curves follow the mean values. Five hundred and eighty larvae of all sizes were used in 17 dipyrindyl oil experiments, or from 22 to 45 larvae in each (average 34.1 per experiment). Seven hundred and forty-three larvae of all sizes were used in 23 nicotine experiments, or 16 to 40 larvae in each (average 32.3 per experiment). For all concentrations of nicotine above 2.7 per cent, commercial nicotine sulphate solution was

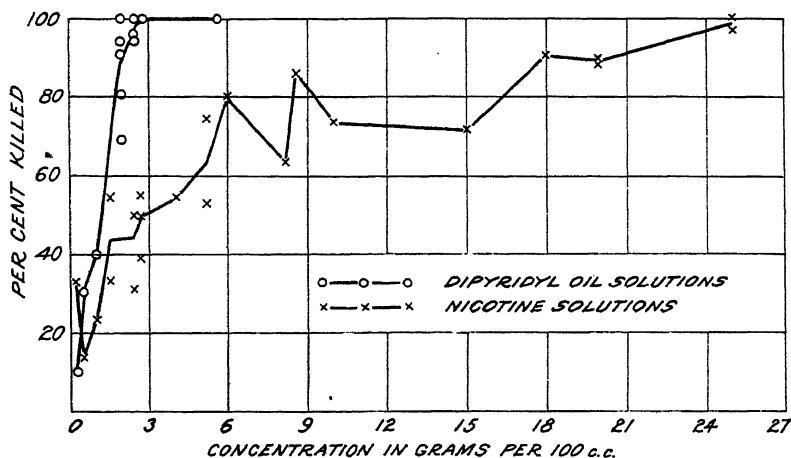


FIG. 3.—Comparison of the toxicity of crude dipyrindyl oil and nicotine to larvae of *Lema trilineata* (submergence experiments)

used. It was diluted to contain nicotine, equivalent by weight to the percentage designated in Figure 3.

All solutions contained 0.3 per cent sodium fish-oil soap. In four experiments with a total of 118 larvae of all sizes the mortality produced by 0.3 per cent soap was 2.5 per cent.

The curve for dipyrindyl oil is abrupt, the percentage of larvae killed rising to 97.4 at a concentration of 2.5 per cent of this mixture. The curve for nicotine rises more gradually and irregularly, and the unusually high concentration of 25 per cent is reached before more than 95 per cent of the larvae are killed. Many of the larvae treated with 4 and 5 per cent solutions of nicotine fed actively on potato foliage after treatment, whereas mixtures of dipyrindyl oil of the same concentrations killed or incapacitated all the larvae treated.

Larvae of *Ephestia kuehniella* were submerged in mixtures of crude dipyrindyl oil. Comparable tests were made with nicotine solutions also. The results of these experiments are shown graphically in Figure 4.

The submergence time in these experiments was 1 minute.

Each point in Figure 4 represents a single experiment; the curves follow the mean values. The dipyriddyil oil experiments involved a total of 332 larvae 7 to 13 mm. in length; 24 to 36 larvae (average 27.7) were used in the individual experiments. For the nicotine experiments, 308 larvae 8 to 13 mm. in length were used, or 31 to 37 (average 34.2) in the individual experiments. All solutions contained 0.3 per cent sodium fish-oil soap. Experiments with this soap solution on 173 larvae gave the low toxicity of 2.9 per cent.

Under the conditions which prevailed in these experiments, dipyriddyil oil was more toxic at all concentrations than nicotine. The highest (3.4 per cent) killed 93.3 per cent of the larvae, as compared with only 54.4 per cent for a 5 per cent solution of nicotine.

Larvae of the silkworm (*Bombyx mori*) were also used in submergence tests to ascertain the relative toxicity of crude dipyriddyil

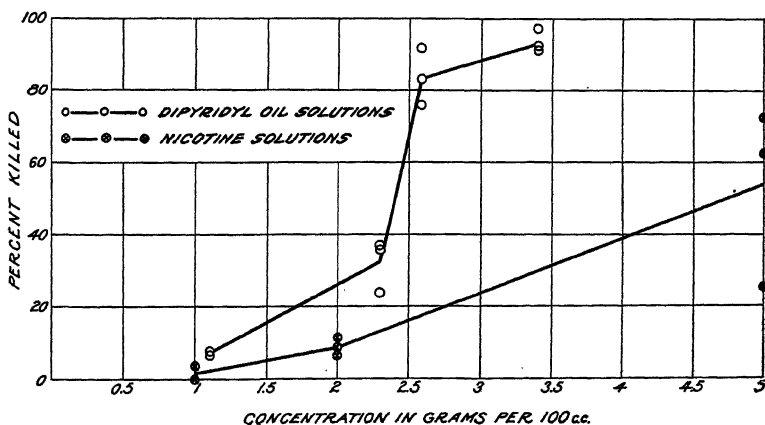


FIG. 4.—Comparison of the toxicity of crude dipyriddyil oil and nicotine to larvae of *Ephestia kuehniella* (submergence experiments)

oil and nicotine. In 1923 the approximate toxic concentration was determined for both of these compounds by the treatment of small numbers of larvae with successive dilutions. Then 85 larvae 25 to 50 mm. in length were divided into four lots and submerged in a 1.5 per cent mixture of crude dipyriddyil oil. From the four experiments an average kill of 97.6 per cent was obtained. In 1924, 100 larvae 30 to 40 mm. in length were treated in four lots of 25 larvae each with a solution of the same strength. The result was a mortality of 96 per cent, in close agreement with the previous determination. Under identical conditions nicotine was more toxic. Thirty-two larvae 25 to 50 mm. in length were used in four tests in 1923, a 0.01 per cent solution killing 96.9 per cent of the larvae. In 1924, of 100 larvae 35 to 50 mm. in length 95 per cent were killed by the same concentration. Some of the larvae of the 1924 experiments, however, probably were diseased, for of 103 control larvae treated with the 0.3 per cent soap solution 19.4 per cent died. Of the 31 control larvae in the earlier experiments (1923) 6.4 per cent were killed or died of disease.

In spite of the small number of larvae available for these experiments the results indicate, it is believed, a significant difference in toxicity in favor of nicotine.

The submergence tests show that the larvae of *Leptinotarsa decemlineata*, *Lema trilineata*, and *Ephestia kuehniella* are more susceptible to crude dipyrindyl oil than to nicotine, but the reverse is true of adults of *L. decemlineata* and silkworm larvae. In view of these results, it seems possible that crude dipyrindyl oil may prove to be more effective than nicotine for the practical control of some injurious insects.

EFFECT OF SPRAYS ON PLANTS

Sprays containing crude dipyrindyl oil have been tested on a variety of plants, under both greenhouse and outside conditions. Nasturtium plants growing in the greenhouse in February and November, 1923, tolerated 0.1 per cent mixtures of crude dipyrindyl oil, a concentration which lies well beyond the minimum toxic concentration (0.05 per cent) for *Aphis rumicis*. At 0.25 per cent concentration the injury to this plant was not severe. Cabbage plants in the greenhouse in April were not injured by mixtures containing 0.05 per cent; higher concentrations were not tried. One-tenth per cent mixtures burned slightly the tips of the fronds of Boston fern, whereas 0.04 per cent mixtures had a very slight effect or none on the young growth (February). Apple foliage and *Coreopsis* plants growing outdoors were not injured by 0.2 per cent mixtures of dipyrindyl oil, and the young growing shoots of rose were unharmed by 0.25 per cent mixtures. All of the above mixtures contained 0.3 per cent sodium fish-oil soap as a spreader.

In every case in which aphid-infested foliage was sprayed, it was possible to kill the aphids without injury to the foliage.

INSECTICIDAL PROPERTIES OF THE CONSTITUENTS OF CRUDE DIPYRIDYL OIL

As stated above, the crude dipyrindyl oil used in this investigation consisted largely of 3 isomers, $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyrindyl, with small quantities of other dipyrindyls and other substances. It has already been shown that $\gamma\gamma$ dipyrindyl has little toxic effect on *Aphis rumicis* (3, p. 4).

In order to study the insecticidal properties of the constituents of the crude oil, a number of preparations were made. Samples of $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyrindyl were obtained which gave constant melting points and were unquestionably of high purity. They were made up in mixtures containing fish-oil soap to compare with the other mixtures used in this investigation. Their toxicities to *Aphis rumicis* when applied as sprays are compared in Figure 5.

The experiments were made in groups of three for each concentration. In the $\alpha\alpha$ dipyrindyl experiments from 707 to 2,249 aphids were used for each concentration; in the $\beta\gamma$ dipyrindyl experiments, 818 to 1,097 aphids; in the $\beta\beta$ dipyrindyl experiments, 851 to 1,103 aphids, except for the 0.05 and 0.1 per cent concentrations, where

349 and 206 aphids, respectively, were used. Each point in Figure 5 is an actual experiment; the curves follow the mean values of each group of experiments.

At the higher concentrations $\alpha\alpha$ dipyridyl appears to be a little more toxic than $\beta\gamma$ dipyridyl, a 0.6 per cent mixture of the former corresponding in toxicity with a 0.75 per cent mixture of the latter. The effect upon nasturtium foliage (under greenhouse conditions) is also different; 0.5 per cent mixtures of $\alpha\alpha$ dipyridyl applied in September and October caused only slight injury, whereas the same concentrations of $\beta\gamma$ dipyridyl applied in October caused severe injury. The tolerance of nasturtium plants for $\beta\gamma$ dipyridyl was reached at approximately 0.1 per cent concentration.

The sample of $\beta\beta$ dipyridyl used in these experiments was synthesized from pseudophenanthroline by oxidation with potassium permanganate. As indicated in Figure 5, it proved to be less toxic than either $\alpha\alpha$ or $\beta\gamma$ dipyridyl, a 1 per cent solution killing only 93.8 per

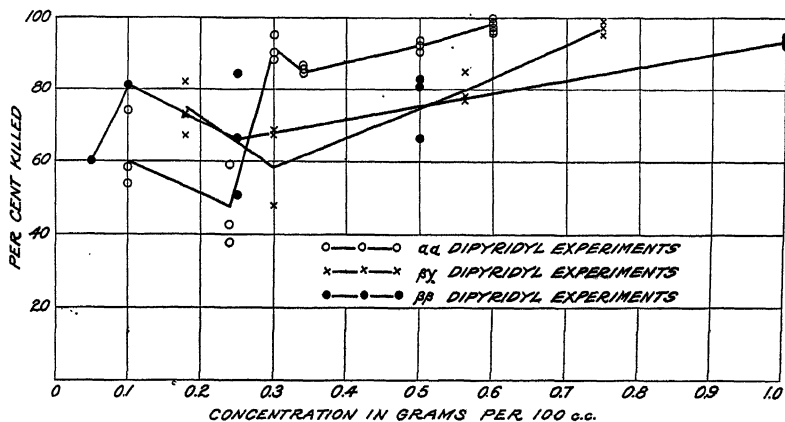


FIG. 5.—Comparison of the toxicity of $\alpha\alpha$ dipyridyl, $\beta\gamma$ dipyridyl, and $\beta\beta$ dipyridyl to *Aphis rumicis* (spraying experiments)

cent of *Aphis rumicis* in these tests. The tolerance of nasturtium plants for this compound in the greenhouse during August was of about the same order as that noted for $\beta\gamma$ dipyridyl.

The preparation of pure $\alpha\beta$ and $\alpha\gamma$ dipyriddyls is under investigation at the present time. An impure sample of $\alpha\beta$ dipyridyl prepared from phenanthroline was much less toxic than crude dipyridyl oil.

From the above experiments it appears that the toxicity of crude dipyridyl oil is not dependent so much upon $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyriddyls as upon a compound or compounds present in the water-soluble fractions. This compound must have high toxicity to aphids, because several preparations which were unquestionably impure have killed about 90 to 95 per cent of *Aphis rumicis* at concentrations lying between 0.05 and 0.01 per cent, a close approximation to the toxicity of nicotine for this aphid. Work is being continued with the hope of isolating and identifying this compound.

INSECTICIDAL PROPERTIES OF OTHER COMPOUNDS

A number of other compounds were tested in the course of this investigation, and are included in Table 2.

TABLE 2.—*Spraying experiments against Aphis rumicis on nasturtium plants*

Compound	Concentrations tested, grams per 100 c. c. ^a	Concentrations necessary to kill 95 per cent or more of the aphids	Concentrations tolerated by nastur- tium plants
Allyl alcohol.....	1, 2.....	>2	<2
Anisole ^b	1, 5, 10, 20.....	20	>1<5
Betahydroxypyridine.....	0.15.....	>0.15	>0.15
Betathionaphthol (commercial).....	0.5, 1 c.....	>1	<0.5
Carvacrol.....	0.25, 0.5, 1, 2.....	1	<0.25
Chloramine T.....	1, 5.....	>5	>5
Chlorocyclohexane.....	1, 5.....	>5	<1
Citral (technical).....	0.25, 0.5, 1, 2.....	2	±0.25
Dimethylaniline.....	1, 5, 6.....	6	>1<5
Isonicotine.....	0.25, 0.5, 1, 1.5, 1.7.....	1.7	<0.25
Methyleyclohexane.....	7.5, 15.....	>15	±7.5
Paracymene.....	4, 5, 7.....	7	<1
Pyridinebetasulphonic acid.....	4.9.....	>4.9	<4.9
Pyrolidone.....	0.1, 0.5, 1, 2.....	>2	0.5-1
Trioxymethylene.....	0.5, 1, 2.....	>2	>2
Urethane.....	1, 2, 2.5, 5.....	5	1.2

^a 0.3 per cent sodium fish-oil soap used as a spreader.

^b Tattersfield, Gimingham, and Morris (6, p. 230) have found that anisole has little toxicity for *Aphis rumicis* at a concentration of 5 per cent.

^c With sufficient NH₄OH to render it soluble.

Several of these are of special interest because of certain similarities to the dipyriddyis or nicotine. Betahydroxypyridine and pyridinebetasulphonic acid, like nicotine, have the substituted group attached at the β carbon. Neither of these compounds, however, showed striking toxicity; like other sulphonic acids (3, p. 6), pyridinebetasulphonic acid was relatively nontoxic. Isonicotine resembles $\gamma\gamma$ dipyridyl in that it is formed by the linkage of two pyridine rings through the γ positions. It differs structurally from this compound in that one ring is hydrogenated to form a piperidine grouping. Although more toxic than piperidine (3, p. 4), it is still much less so than crude dipyridyl oil. None of the other compounds showed striking toxicity.

SUMMARY

A crude oil containing several isomeric dipyriddyis and other substances has been prepared from pyridine and sodium. The physical properties and chemical relationships of the dipyriddyis are discussed and their similarities to pyridine and nicotine pointed out.

In spraying experiments with six species of aphids, this crude dipyridyl oil was found to be highly toxic, but was surpassed in this respect by nicotine.

In submergence experiments, the crude oil was more toxic than nicotine to the larvae of two species of Coleoptera and one species of Lepidoptera, and less toxic than nicotine to the adult of one species of Coleoptera and to silkworm larvae.

Crude dipyridyl oil, in the present experiments, was not injurious to plants infested with aphids at concentrations sufficient to kill the aphids.

$\alpha\alpha$, $\beta\beta$, $\beta\gamma$, and $\gamma\gamma$ dipyridyls, which occur in the crude dipyridyl oil used in these tests, were not so toxic to *Aphis rumicis* as the crude oil itself; $\gamma\gamma$ dipyridyl is much less toxic than the other three compounds.

Several preparations from the crude oil proved to be highly toxic to *Aphis rumicis* on nasturtium plants. Although unquestionably impure, they approached closely the toxicity of nicotine for this aphid. Work is being continued on these preparations.

The toxicities of 16 other organic compounds tested during this investigation are given also (Table 2). Several of these are related to the dipyridyls or to nicotine. As compared with dipyridyl oil, they showed no appreciable toxicity.

Crude dipyridyl oil was more toxic than nicotine to certain insects used in this investigation, and it is possible that it may also prove more effective for the practical control of some injurious insects.

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AGE CLASSES OF WESTERN WHITE PINE PLANTING STOCK IN RELATION TO ASPECT OF PLANTING SITE IN NORTHERN IDAHO ¹

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INTRODUCTION

In the northern Rocky Mountain region there are vast areas of forest land denuded by fire, which will remain virtually unproductive for generations to come unless planted by hand with nursery-grown trees. After the first sweep of fire through the original stands of western white pine timber (*Pinus monticola*) on these lands, the forest in most instances started to come back naturally, but before recovery was complete fire came again and this time swept away all the small new trees and all sources of seed for future growth. Forest planting is the only practical means of making such land productive.

Because of the vast amount of forest planting to be done in this region, it is desirable that research point out the means by which it can be undertaken at smallest cost and with the best possible results in thrifty young trees. This paper is an account of a few experiments to that end, other studies with a bearing on survival of plantations being still under way.²

Two very important factors in forest planting are the aspect³ or exposure of slopes used for planting and the class of stock employed, for these are factors which both singly and in combination have a definite effect on survival of the trees. In the experiments reported herein five classes of stock representing three different ages of trees were tested on each of four slopes. The exposure of the slopes was determined in each instance by averaging the compass readings on the plots of each series. The slope classed as northeast had no deviation from this direction and the northwest, east, and west did not vary greatly, the first being 14° north of due northwest and the last two 8° north of east and 2° south of west, respectively.

The topography was such that the four aspects studied could be found close together, each with a gradient of 25° to 30° and at practically the same elevation, 3,350 to 3,700 feet. It is essential in work of this kind to arrange test plots so as to minimize irregularities in uncontrolled influences, such as differences in the competing vegetation, variation in the direction of slope, and changes in the quality of soil within plots and between plots. The soil, for instance, is often deeper and more favorable for plant life near the base of a slope than higher up. In these experiments the plots on each slope ran parallel to each other up and down the slope, each containing 600 trees of a certain age class set in 8 rows. Because of the uniformity of the

¹ Received for publication Apr. 1, 1926; issued October, 1926.

² The experiments on which this report is largely based were started by E. C. Rogers near Wallace, Idaho.

³ "Aspect," as used by foresters, denotes the direction toward which a slope faces.

sites as a whole and the narrowness of the plots used, there is every reason for confidence in the results obtained.

The slit method of planting was used. This method is rapid and requires but one man for the entire operation, the soil being worked with a short-handled mattock and the seedlings planted with the free hand.

The crew for the experimental plantings consisted of a foreman, eight planters, and a packer. Each planter set out one row of trees in each plot, so that the method of planting might be considered uniform. To make later observations and records easier, each plant was marked with a serial number on a white-tipped stake.

The planting was done in the spring of 1915 with age classes of nursery stock designated as 2-2, 1-2, 2-1, 2-0, and 1-1.⁴ At the end of the dry period in the fall of 1915, 1916, and 1917, all plants were examined, the surviving plants were counted, and the current stem-height growth of 200 plants in each plot was measured to the nearest tenth of an inch. The measured plants were chosen from all parts of the plot, upper as well as lower ends, the selection of 25 plants from each row insuring an equal representation of the work of each planter. As nearly as possible, the same plants were measured each year. Changes in the list of trees measured the first year were made only where necessary in order to confine the observations on growth to normal, uninjured plants. This was done because mechanical injury bears no obvious relation to the age class of the trees or to the exposure of the planting site.

SURVIVAL BY AGE CLASSES

A study of the percentage of planted trees surviving through three seasons, as given in Table 1, makes apparent certain relationships.

TABLE 1.—*Survival percentages of western white pine planting stock by age classes, aspects, and seasons*^a

Age class	Northwest			Northeast			East			West			Average		
	1915	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917
2-2-----	100.0	99.4	99.1	98.7	98.6	97.6	99.2	98.3	98.2	97.3	93.2	87.4	98.8	97.4	95.6
1-2-----	99.5	98.8	98.8	99.8	98.7	99.0	99.4	98.8	98.4	95.9	92.2	80.7	98.7	97.1	94.2
2-1-----	98.4	96.7	95.8	99.0	97.6	96.7	98.4	96.6	95.9	95.0	87.9	77.4	97.7	94.7	91.4
2-0-----	97.5	96.3	94.6	98.4	96.0	91.7	99.3	98.6	97.5	91.0	80.5	69.4	96.5	92.8	88.3
1-1-----	97.6	95.0	93.5	96.1	90.1	88.0	95.1	90.8	88.1	83.8	75.4	63.1	93.2	87.8	83.2
Average-----	98.6	97.2	96.4	98.4	96.2	94.6	98.3	96.6	95.6	92.6	85.8	75.6	97.0	93.9	90.5

^a Trees planted in May, 1915, on the Placer Creek area near Wallace, Idaho.

It may be seen from the average survival for all aspects that 2-2 stock was most successful, with 1-2, 2-1, 2-0, and 1-1 following in descending order. This order held for the favorable northwest aspect, and even more clearly for the west aspect, where survival in general was lower. On the slopes facing northeast and east the 2-2

⁴ As readers familiar with nursery practice will know, this terminology indicates the number of years spent by the plant in the seed bed and in the transplant bed. For example, the 2-2 stock remained two years in the seed bed and two years in the transplant bed, and was 4 years old at the time of field planting. Similarly, 1-2 stock and 2-1 stock were both 3 years old, but the former was transplanted at 1 year of age the latter at 2 years. The 2-0 stock was seedlings 2 years old used in the field without previous transplanting.

stock yielded first place to the 1-2 stock, but only by small margins. The 1-1 stock was poorest on all aspects.

This similarity of the relative survival of age classes for different aspects gives added meaning to the figures for average survival as an indicator of quality of stock. The intrinsic qualities that enable one class of stock to survive better than another have not been definitely determined. Differences in survival, however, are thought to arise not only from variation in size and vigor as a result of difference in age, but also from variation in anatomical proportion resulting from different cultural treatment in the nursery. Thus 1-2 and 2-1 stock or 2-0 and 1-1 stock, although the same age, differ anatomically because of the different times at which they were pruned and transplanted in the nursery. The survival of 3-year-old trees was intermediate between the 2-year-old and 4-year-old trees, suggesting a general tendency toward higher survival with increased age. This tendency is not proved, however, because in this study the influence of age can not be separated from that of class of stock.

Aspect of planting site is as important a factor in survival as is class of stock, for reforestation of any area can be successful only when growing conditions there meet the requirements of the trees planted. Severity of site, as indicated by differences in survival, also emphasized the differences attributable to quality of stock. The west slope sites were clearly the most severe. The other, more moderate sites, were about equally favorable except that mortality was slightly less on the northwest slope than on the other two.

Assuming that all readily avoidable causes of death were eliminated from the tests, the above comparison on the basis of numbers of trees surviving gives a conception of relative values of classes of stock and aspects of planting site. Such an assumption, however, would not be justified in the absence of observations on the causes of mortality. For this reason all dead trees were dug out and inspected at the annual fall examination.

For most of the trees no reason for death other than drought could be detected, but occasional evidence of other causes was found. Some roots had been pruned too short, some trees had bark stripped from their roots, and others had scraped stems. These three causes can be kept at a minimum by careful handling of trees and the culling out of injured individuals. Shallow planting, whether resulting from roots cut too short or from trees set with their roots in poor positions, caused death because the surface layers of soil dried out most rapidly. Deep planting may have resulted in poor aeration for roots and may have buried part of the tops. Plants set under large fallen logs had a poor chance to live. Even after heavy September rains it was noticed that the soil in such places was dust-dry and often used by grouse for wallows. Still more fatal was the planting in what seemed to be fairly deep soil on the root mass of wind-thrown snags.

The most frequent cause of death listed other than drought was sliding soil on steep slopes. This is not to be confused with the rapid and much more destructive mass movement of soil, logs, and other debris caused by one of the snow slides common in the region, but is rather a creeping of soil, small rocks, and other surface litter from above the trees downward, usually caused by rain and melting snow. The sliding soil has a tendency to fill up the slight depression formed

by planting and to bury the trees. Death of trees resulting from covering by sliding soil naturally fell heaviest on the youngest and shortest-stemmed trees, the 1-1 stock.

These younger trees seemed also to have been more subject to stem injury from tamping. The older trees were probably bruised as often, but were better able to withstand the injury. Similarly, by far the largest number of dead trees showing evidence of either mechanical injury or poor planting were on the west-facing slope. Such unfavorable conditions were probably as frequent on other aspects, but there greater moisture enabled the trees to live in spite of adverse conditions.

Less than 1 per cent of the trees planted, or 10 per cent of those which died, were affected by the above-mentioned unfavorable conditions, leaving 90 per cent of the deaths attributable only to drought. As has been indicated, the older plants usually survived better than the younger and the transplants better than the seedlings.

The conclusion that transplants are hardier than seedlings is corroborated by the results of small tests of age classes at other points. In one of these tests at Priest River, Idaho, in the spring of 1915 where 2-0 and 1-2 western white pine stock were planted on a flat and on a northeast slope, the transplanted stock (1-2) yielded higher survival in both situations. In another test near Haugan, Mont., in the fall of 1922, parallel trials of 3-0 and 1-2 white pine stock on a northwest slope yielded higher survival again for the transplanted (1-2) stock. Also a plantation on a northeast slope in the fall of 1924 resulted in higher survival for 1-2 transplants than for 3-0 seedlings. In this plantation 2-0 trees were inferior to both of the other classes of stock.

Success in planting in the region as a whole is very largely dependent upon adequate rainfall, especially during the first year or two after planting, and this was shown in the experiments made in the present study. The nearest weather station is at Wallace, Idaho, about 3 miles away and several hundred feet lower in elevation. The Weather Bureau records there indicated more than the average amount of rainfall in July and August for 1915 and 1916, the first two years after planting. Had this planting been done in the spring of 1917 or at any time during the next four years the results might have been quite different, for these were years of severe summer drought. Experiments in which the outcome is judged mainly by relative survival are likely to fail in such dry years, unless placed on the very best sites, because of universally high mortality. In like manner it has been observed that such experiments in moist years are often not very instructive for the opposite reason—mortality is so universally low that clear distinctions can not be made, except perhaps on the driest sites. Unfortunately, so far as the forests are concerned, drought can neither be controlled nor its occurrence predicted. Knowledge of drought hardiness must be accumulated year by year in the course of various observations on forest planting material.

HEIGHT GROWTH

Figure 1 shows the average elongation of stems or height growth of terminal shoots during the period of study.

It may be deduced from these measurements that the older the plant the greater is the height growth of stem. In general, the

growth of the 3-year-old trees (2-1 and 1-2 stock) was intermediate between the younger (1-1 and 2-0) and the older (2-2) trees each year. This relationship is especially noticeable in the cumulative effect of three years' growth. That the older plants grew faster than the younger is shown not only by a comparison of growth during any one year, but also by the accelerated growth of any one class of stock during the three years. Acceleration of height growth over a period of years is a matter of common observation on somewhat older seedlings.

It appears from Figure 1 that on all aspects the height growth of 2-0 stock in any given season was less than that of 1-2 stock. However, considering the fact that the transplanted stock was 1 year older than the seedlings, might it not be fairer to contrast the growth of 1-2 stock the first and second seasons after planting with that of 2-0 stock during the second and third seasons? An objection may be made that the seedlings had more time than the transplants to

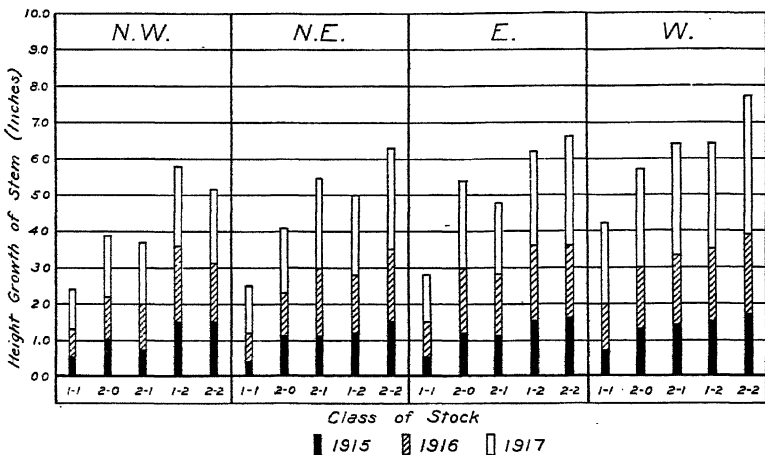


FIG. 1.—Height growth of stems of western white pine planted in the spring of 1915 on the Placer Creek area, Wallace, Idaho

recover from the shock of planting, but this objection would hardly apply to both the second and third years. Also, as stem growth occurs during the spring and early summer, it should not have been greatly influenced in this case by summer drought, especially as the months in question were above normal in amount of precipitation.

If this standard of comparison be accepted, and the growth data be charted as in Figure 2, it is evident that the 2-0 stock is growing at rates slightly faster on an average than those of the 1-2 stock. Also, by the same standard 1-2 stock is growing relatively faster than 2-2 on all aspects, making the slightly higher survival of the latter seem of less consequence. The significance of this comparison will be more apparent when relative planting costs are discussed.

Aspect also had a pronounced effect on growth, and the same trends are shown during each season and by each class of stock. The fastest stem growth took place on the west slope, then on the east, northeast, and northwest slopes in decreasing order. The

average growth of the planted stock during the 3-year period following planting was nearly 2 inches greater on the west slope than on the northwest, whereas the east and northeast slopes led the northwest by an inch and a half inch, respectively. Thus it may be seen that the poorest survival was accompanied by the most rapid growth, and vice versa.

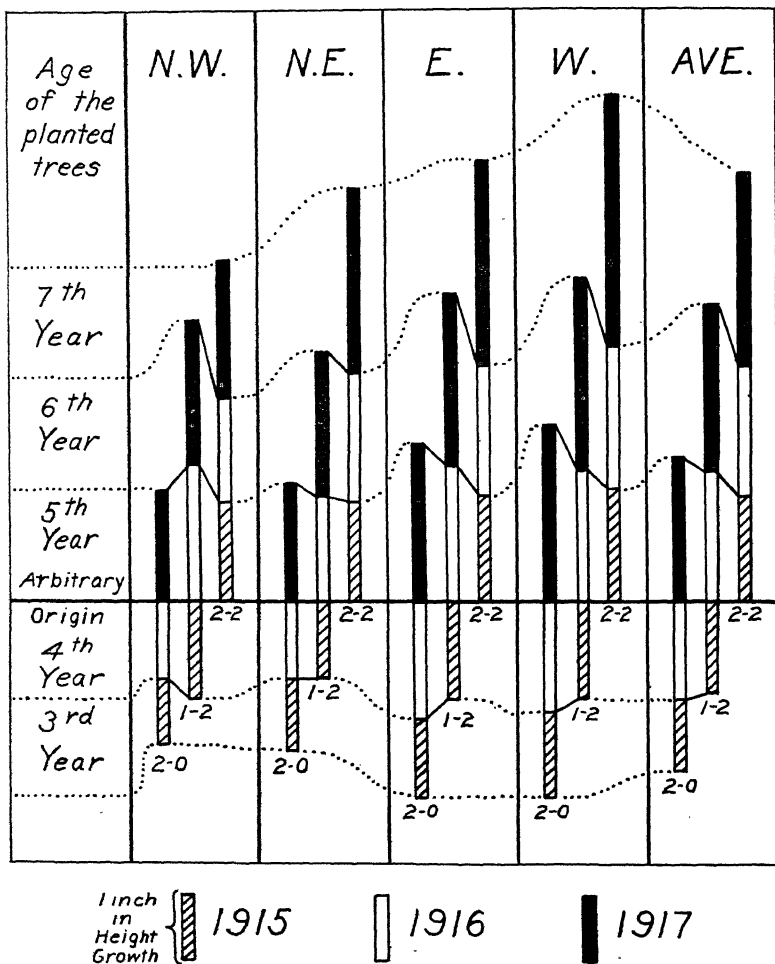


FIG. 2.—Comparison of growth of trees of same age. Stem height growth of different classes of stock during the first three years after planting

A somewhat obvious explanation of this opposite action on survival and on growth proves fallacious on analysis. Because considerable variation in development exists between individual plants of any class of stock, it seems at first glance reasonable to suppose that on drier sites only the superior plants survive, and consequently raise the growth averages for these sites above those taken on moist sites, where natural selection is less severe. In other words, it might be

reasoned that, when all survivors are considered, the quality of plants left after natural selection would be higher on the drier than on the moister sites, and that the difference might well be sufficient to account for the greater growth on drier sites.

This supposition will not "hold water," however, when a very simple test is applied. Inasmuch as survival of the different classes of stock on the moist northwest slope was fairly uniform and a wide divergence in the survival of these classes appeared on the dry west slope, it should follow that the stock on the west slope that survived least well, namely, the 2-0 and 1-1 lots, would show the greatest relative advance in height growth over that on the northwest aspect, for with them selection was most severe. However, when the decrease in survival for each class on the west slope below that on the northwest slope is expressed in percentages and compared directly with the percentage increase in growth on the west over the northwest, no consistent relation obtains. This is shown clearly in Table 2. Furthermore, the rather slight differences in survival on the northwest, northeast, and east slopes are in contrast to striking differences in growth. The hypothesis that natural selection following planting determines the growth rates on these sites does not seem tenable.

TABLE 2.—*Comparison of growth and mortality relations on northwest and west aspects three years after planting*

Age classes	Decrease in survival percentage on west aspect below that on northwest aspect	Per cent increase in growth on west aspect over that on northwest aspect
2-2.....	11.7	48.1
1-2.....	18.1	27.6
2-1.....	18.4	73.0
2-0.....	25.2	41.0
1-1.....	30.4	75.0

In seeking a truer explanation, a little speculation may be justified. In the complex interaction of environmental influences it may well be that light has a dominant rôle in the determination of the rate of growth on these slopes. The northwest slope receives but little more than diffuse light, whereas the west slope is exposed to direct sunlight in summer during the greater part of the day. Although little or nothing is known as yet of the optimum light intensity for synthesis of plant food—for assimilation, for cell division, and stem elongation of western white pine during its early development—it is possible to estimate from the figures for mortality from drought that transpiration, as well as evaporation from the soil, was greatest on the west aspect and least on the northwest. If, as is sometimes held, faster transpiration is accompanied by faster absorption of nutrient salts, sunlight may have been a controlling influence in the growth differences observed.

COST

Survival and growth have been considered in an effort to judge the relative merits of different age classes of planting stock and different aspects for planting sites. The cost per thousand of established trees is a better criterion where it can readily be determined. Cost is a factor that can never be disregarded in practice.

In comparing planting stock on the basis of survival costs it must be remembered that only relative differences for single years are comparable, because the cost of production and planting of tree stock fluctuates greatly from year to year with the price of labor and supplies. The same relative differences in cost should be fairly constant from year to year, however. The nursery costs shown in Table 3 are based on nursery practice and labor conditions existing in the spring of 1918 and include all expenses from seed collection to loading tree shipments on the train. The field cost is the same for all age classes and is taken from the cost of planting 1-2 white pine on the St. Joe National Forest in the spring of 1917. It includes shipping charges for nearly the same distance as for the stock used on the Wallace area. An obvious imperfection in this method of figuring lies in the fact that the smaller-sized trees can be both shipped and planted somewhat more cheaply than the larger. By neglecting these things a slight burden will be placed on the 2-0 and 1-1 stock and a corresponding advantage given to the 2-2 stock. The cost of survival may be computed by the following formula:

$$\text{Cost per 1,000 survivors} = 100 \times \frac{\text{total cost of planting 1,000 trees}}{\text{percentage of survival}}$$

Substitution in this formula of figures given in Tables 1 and 3 gives the data for Table 4.

TABLE 3.—Costs of planting per 1,000 trees

Age class	Nursery	Field	Total
2-2.....	\$3.64	\$9.99	\$13.63
1-2.....	3.28	9.99	13.27
2-1.....	3.06	9.99	13.05
2-0.....	1.67	9.99	11.66
1-1.....	2.70	9.99	12.69

The data in Table 4 indicate clearly that for an equal investment in planting work more living trees were obtained with 2-0 stock than with any other age class tested when planting was done on favorable aspects such as the northwest, northeast, and east, and this advantage would have been accentuated had it been possible to consider in the figures the somewhat cheaper shipping and planting charges for this class of stock. On the severe west aspect the oldest stock, the 2-2, appears to have been less expensive, although when allowance is made for its having been perhaps unduly favored in the cost figures, the margin between the 2-2 and the 1-2 classes of stock may be considerably reduced. In general, 2-0 was least expensive, 1-1 most expensive, and the three older transplant classes were intermediate and a great deal alike. The universally high cost on the west aspect is due, of course, to the high mortality on that exposure.

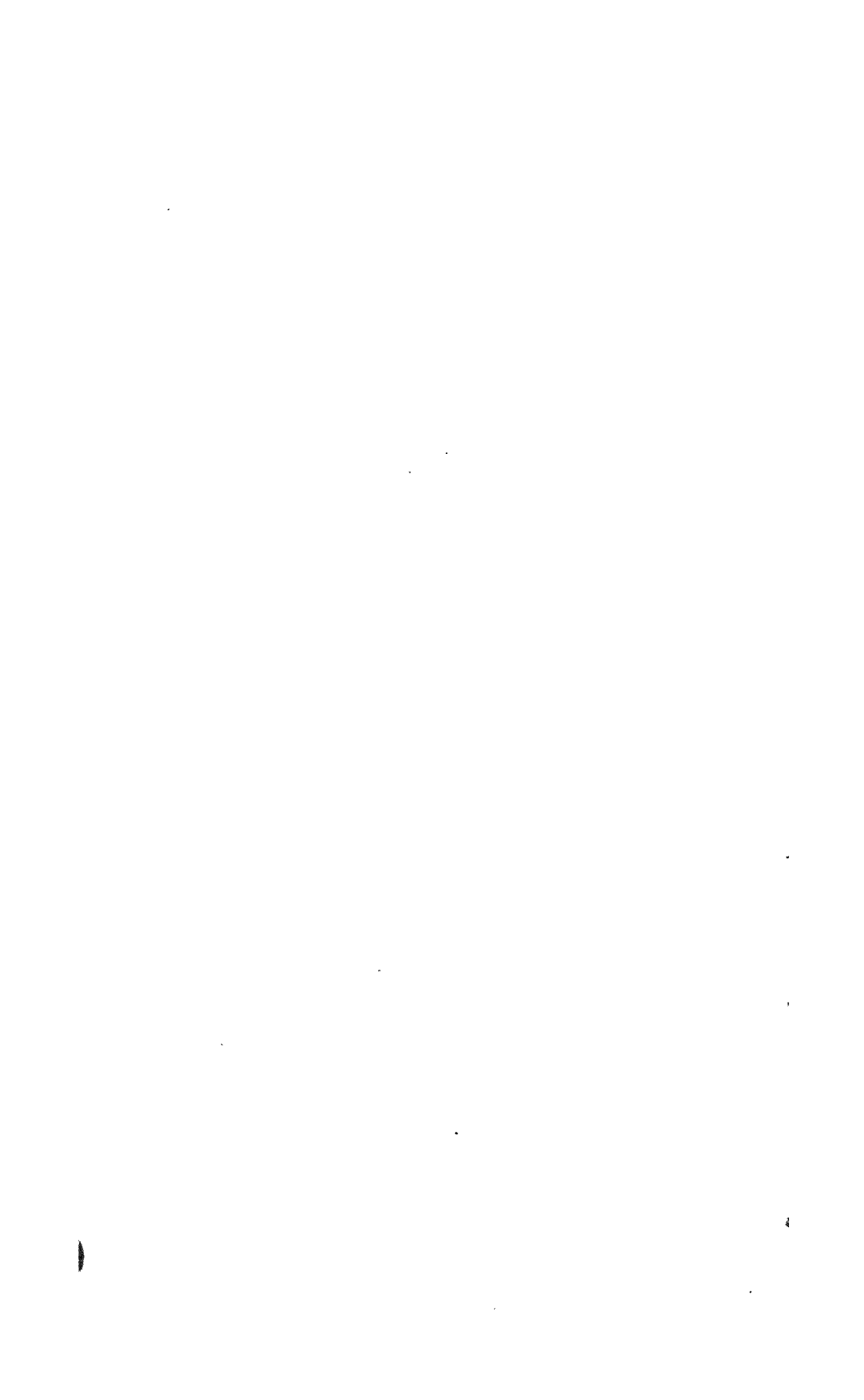
TABLE 4.—*Cost per thousand trees surviving three years after planting on different aspects and with different age classes*

Aspect	Age classes					Average
	2-2	1-2	2-1	2-0	1-1	
Northwest.....	\$13.75	\$13.43	\$13.62	\$12.33	\$13.57	\$13.34
Northeast.....	13.97	13.40	13.49	12.71	14.42	13.60
East.....	13.88	13.49	13.61	11.96	14.40	13.47
West.....	15.59	16.44	16.86	16.80	20.11	17.16
Average.....	14.30	14.19	14.39	13.45	15.62	-----

SUMMARY

In general the survival of planted western white pine trees places 2-2 transplanted stock at the top, with 1-2, 2-1, 2-0, and 1-1 following in descending order. Height growth also conforms to this order. The cost of survival definitely favors 2-0 seedlings on moderate sites, and 2-2 or 1-2 only on the more severe sites.

Aspect of planting site is as important as the class of stock. Of the four exposures tested the lowest survival, accompanied by the fastest growth, occurred on the west exposure. The east, northeast, and northwest aspects showed considerably higher survival in the order given.



THE PERFECT STAGE OF THE FUNGUS WHICH CAUSES MELANOSE OF CITRUS¹

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INTRODUCTION

A disease of citrus and related plants to which the common name melanose is applied was first recognized near Citra, Fla., by Swingle and Webber² in 1892. Their account of the disease, published in 1896, states that in their opinion it was caused by a "vegetable parasite" which they were not able to isolate in culture. In 1912 a paper by Fawcett³ was published in which he set forth the results of his investigations on a type of stem-end decay of fruits, and he ascribed the cause of the decay to a previously undescribed organism which he designated *Phomopsis citri*. The relationship between this stem-end rot and melanose was not suspected at first. Evidence has been presented by Floyd and Stevens,⁴ however, and by others who have investigated this problem, which shows that the two forms are undoubtedly caused by one and the same fungus. The rules of proof to establish this relationship have never been completely followed, because thus far it has not been possible for anyone to isolate *Phomopsis citri* from melanose lesions on leaves, twigs, and fruits.

In July, 1925, the present writer found, on fallen decaying twigs of lime (*Citrus aurantifolia* Swingle), on the grounds of the United States Citrus-Disease Field Laboratory, Orlando, Fla., a species of Diaporthe. Since several species of the form genus *Phomopsis* are known to have an ascigerous stage belonging to the genus *Diaporthe*, it was suspected that these specimens were those of the perfect stage of *Phomopsis citri*. Subsequent collections of the perfect stage were made in August and September from twigs of grapefruit (*Citrus grandis* Osbeck), sweet orange (*Citrus sinensis* Osbeck), and tangerine (*Citrus nobilis* Lour.), in the vicinity of Plymouth, Winter Park, and Gotha, Fla. A bundle of affected twigs bearing the *Phomopsis* stage was collected in September and placed in a sheltered location in contact with the ground. It was examined from time to time to watch the development of the perfect stage. During the last week in the following January it was found that perithecia had developed to maturity on the twigs.

These field observations when taken collectively indicate that the *Diaporthe* stage may be found at any time throughout the year on fallen citrus twigs.

¹ Received for publication March 2, 1926; issued October, 1926.

² SWINGLE, W. T., and WEBBER, H. J. THE PRINCIPAL DISEASES OF CITROUS FRUITS IN FLORIDA. U. S. Dept. Agr., Div. Veg. Path. Bul. 8: 33-38, illus. 1896.

³ FAWCETT, H. S. THE CAUSE OF STEM-END ROT OF CITRUS FRUITS (*PHOMOPSIS CITRI* N. SP.). Phytopathology 2: 109-113, illus. 1912.

⁴ FLOYD, B. F., and STEVENS, H. E. MELANOSE AND STEM-END ROT. Fla. Agr. Expt. Sta. Bul. 111: 5-16, illus. 1912.

ISOLATIONS

Two methods were employed in isolating this *Diaporthe*. Perithecial stromata were crushed in drops of sterile water on a slide, and loopfuls of the suspension were spread over the surface of hardened agar plates. The resultant growth consisted of colonies of *Diaporthe* and several other fungi. Transfers of selected colonies were made to potato-dextrose agar slants and several strains of *Diaporthe* in pure culture were obtained by this means. Much more satisfactory results, however, followed

the inversion of hardened agar plates over twigs placed on strips of moistened blotter in the tops of Petri dishes. The ascospores were forcibly ejected and could be seen to have lodged on the surface of the agar, when examined under the low power of the microscope. The position of individual ascospores was marked on the bottom of the plates and their development noted at intervals. By the use of these inverted plates contaminations were avoided and pure cultures were readily obtained. Several cultures of the fungus were isolated by this method, in August and September, from collections of this *Diaporthe* stage on twigs of grapefruit, sweet orange, and tangerine. The mycelial growth of these several cultures on potato-dextrose agar slants resembled

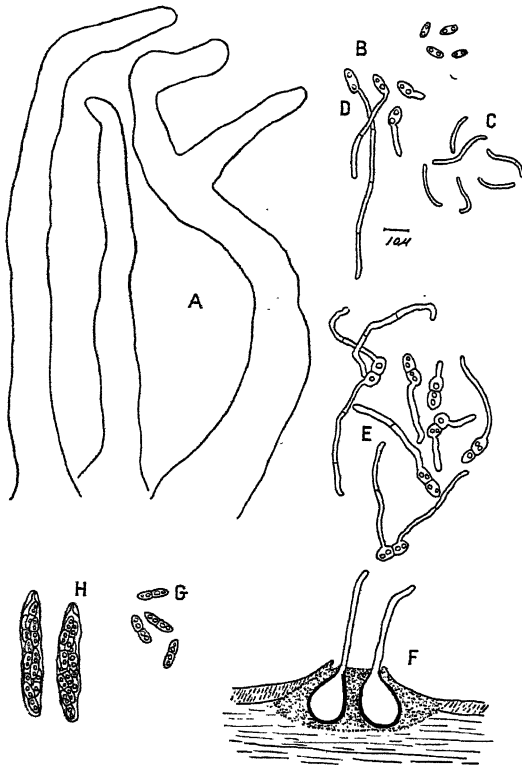


FIG. 1.—A, diagram shows shape of perithecial breaks; B, conidia of *Phomopsis citri* from ascospore cultures; C, stylospores of *P. citri* from cultures from ascospores; D, germinating conidia of *Phomopsis* stage; E, germinating ascospores of *Diaporthe citri*, after growth for 16 hours; F, diagram of stroma and perithecia of *D. citri*; G, ascospores of *D. citri*; H, asci of *D. citri*

that of stock cultures of *Phomopsis citri* isolated from fruit affected with stem-end rot. Since *Phomopsis* forms pycnidia sparingly or not at all on this substratum, subcultures were made on sterilized stems of pigeon pea (*Cajanus indicus* Spreng). In three to four weeks pycnidial production occurred in abundance on this substratum. These pycnidia were of the *Phomopsis* type and bore both functional conidia (fig. 1, B) and stylospores (fig. 1, C). Comparative measurements were made of pycnidia, conidia and stylospores of *Phomopsis citri* from cultures isolated from stem-end rotted fruits, and of similar structures in the cultures from ascospores, and they were found to be similar in every respect.

All cultures, except two which were obtained from ascospores, developed the conidial stage. These two, one of which was cultured on potato-dextrose agar and the other on pigeon-pea stems, were maintained under identical conditions and they produced perithecia (fig. 2). However, subcultures from these in turn developed the pycnidial stage. No adequate explanation can be given at this time to account for the production of perithecia by these two isolations. This problem is especially puzzling because thousands of isolations of *Phomopsis* from decaying fruit have been made during the last few years in the routine investigations in this laboratory and none has been found to develop perithecia.

THE ASCIGEROUS STAGE

The perithecia of this *Diaporthe* can be distinguished from most other fungi by the presence of slender black beaks which protrude singly or in small groups from the cortex of the twigs (fig. 2). A somewhat similar and related organism, *Eutypella citricola* Speg., which appears to be quite widely prevalent in Florida, also has these characteristics and may be confused with *Diaporthe* if one depends entirely upon examination with the unaided eye. Such at least was the writer's experience with collections made at De Land, Fort Pierce, Haines City, Winter Haven, Bartow, Fort Myers, Wauchula, Lakeland, and Sarasota, Fla., near all of which places melanose is prevalent. Further chance for confusion arises from the fact that *Eutypella* may occur interspersed with the citrus *Diaporthe*.

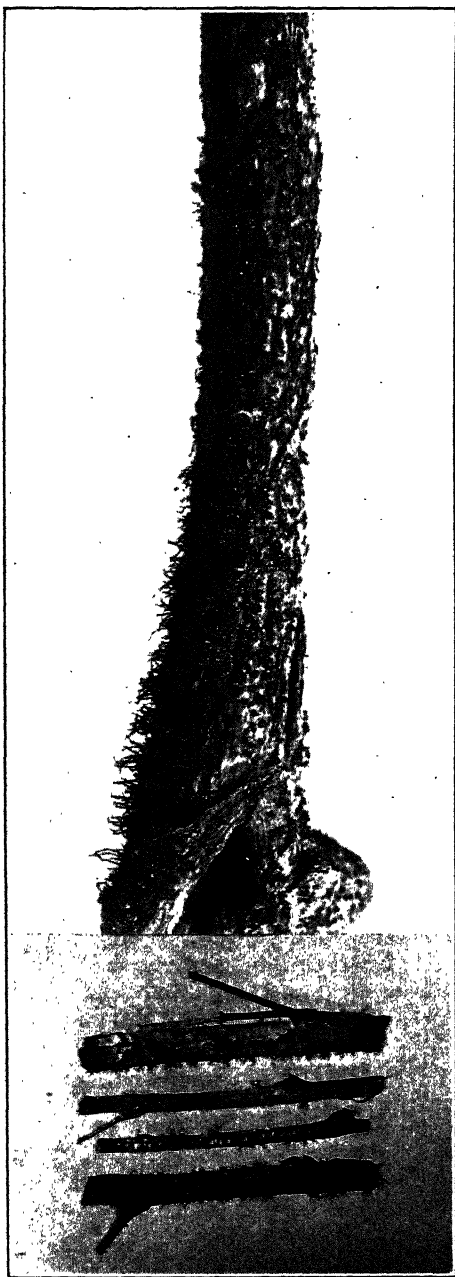


FIG. 2.—Perithecia of *Diaporthe citri* on grapefruit twig (above); and in culture on pigeon-pea stems (below)

The perithecial stromata of *Diaporthe* occur within the bark and remain covered at maturity. The beaks vary in length from 200 to 800 μ , and in diameter from 40 to 60 μ (fig. 1, A). They are bluntly rounded at the apex and are entirely black, except the extreme tip, which is dark brown. The perithecia range from 125 to 160 μ in diameter and are embedded in a black stroma (fig. 1, F). The asci are sessile, elongate-clavate, 50 to 55 μ by 9 to 10 μ . They are thickened at the apex (fig. 1, H), and open by an apical pore. The ascospores are two-celled, slightly constricted at the septum, and each cell typically contains two prominent oil globules (fig. 1, G). They are hyaline, elongate-elliptical to spindle-shaped, and vary from 11.5 to 14.2 μ by 3.2 to 4.5 μ .

The ascospores germinate readily in tap water and on potato-dextrose agar. There is first a considerable increase in volume of the spore, which is followed by the emergence of a single germ tube or one from each cell (fig. 1, E). The mycelium soon becomes septate, and after 48 to 72 hours' growth the colonies are evident to the unaided eye.

INOCULATIONS

Because of the results of the comparative cultural studies, inoculations were attempted with cultures which were bearing pycnidia and which had been isolated from ascospores as the source of inoculum. Swabs of cotton were dipped in conidial suspensions and placed on tender leaves and twigs. These were then protected against desiccation by being wrapped for 24 to 36 hours in waxed paper, and after this time the wrappings were removed. Grapefruit and sweet-orange seedlings which were free from disease were employed in two of the tests. Young flushes of growth on large grapefruit trees were employed in another trial. Infections were evident in about a week, and within 12 to 14 days typical melanose lesions had developed on all inoculated parts. Near-by seedlings which remained free from melanose and uninoculated flushes which remained free served as controls.

Cultures from ascospores were used also to inoculate mature oranges. The surfaces of the fruits were first disinfected by washing with a 1:1000 solution of bichloride of mercury. The inoculum was then introduced through punctures in the sides, after which the fruits were placed in moist chambers. Fruit which was punctured but not inoculated served as controls. Several of the oranges, both inoculated and uninoculated, developed stem-end rot from natural infection around the stems. Infection developed around the punctures of all inoculated fruits, but not around any of those which were uninoculated.

TAXONOMY

The genetical relationship of the *Diaporthe* in question and *Phomopsis citri* is established both by the comparative cultural studies, as reported above, and by the pathogenicity of cultures from *Diaporthe*. Since this relationship is shown for the first time by these studies, and as the specific name *citri* was first employed for this organism, the name *Diaporthe citri* (Fawcett) n. n. becomes the appropriate binomial. Even though accounts by several other investigators contain the descriptive features of the *Phomopsis* stage, it appears best to summarize the characteristics of both stages of the fungus as follows:

Diaporthe citri (Fawcett) n. n.

Syn. *Phomopsis citri* Fawcett.

Ascogenous stage: Perithecia single or in groups, immersed in black stromata which are covered by the bark, 125 to 160 μ in diam.; beaks black, tapering, 200 to 800 μ in length; asci sessile, elongate-clavate, 50 to 55 μ by 9 to 10 μ , apex thickened and pierced by a narrow pore; ascospores hyaline, two-celled, constricted, elongate-elliptical to spindle-shaped possessing four guttulae, 11.5 to 14.2 μ by 3.2 to 4.5 μ .

Hab. Saprophytic on decaying twigs of *Citrus grandis*, *C. nobilis*, *C. sinensis* and *C. aurantiifolia*.

Conidial stage: Pycnidia on dead branches and decaying fruit, scattered, dark, ovoid, thick-walled and parenchymatous, erumpent at maturity, 200 to 450 μ in diam.; conidia ovate to elongate, hyaline, guttulate, 5 to 9 μ by 2.5 to 4 μ , mostly 6 to 8 by 3 μ ; stylospores slender, hooked, 20 to 30 μ by 0.75 to 1.5 μ .

Hab. Saprophytic or parasitic on leaves, stems, and fruits of *Citrus* and related plants. It attacks young leaves, stems, and fruits, causing melanosae; and on mature citrus fruits causes stem-end rot.

SUMMARY

The perfect stage of the organism which causes melanose and stem-end rot of citrus has been found to occur upon decaying twigs of lime, grapefruit, sweet orange, and tangerine.

Its connection with *Phomopsis citri* has been established by growth of both the pycnidial and ascogenous stages in culture from isolations from ascospores. Further, the conidia from cultures from ascospores have been employed successfully in producing melanose lesions on leaves of grapefruit and sweet orange and a rot of mature oranges.

The ascigerous stage belongs to the genus *Diaporthe*, and the binominal *Diaporthe citri* (Fawcett) n. n. is herein proposed.

THE RELATION OF STORAGE TO THE QUALITY OF SWEET POTATOES FOR CANNING PURPOSES¹

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INTRODUCTION

In an earlier paper (10)² the writers reported the results of studies on the sweet potato (*Ipomoea batatas*), dealing particularly with the canning quality of different varieties and giving some attention to methods of handling as affecting the quality of the canned product. Although the data presented therein dealt rather fully with the comparative merits of the different varieties when canned after the usual curing and storage period, that portion dealing with the significance of their behavior under differing conditions of treatment was suggestive rather than conclusive. The number of varieties studied in this connection was limited to four of the well-known sorts, and the chemical data based thereon were, accordingly, incomplete. The sweet potato is known to vary considerably in its composition, and with the results of but two seasons' work in hand, further information as to the constancy of the varietal differences noted would seem to be of considerable value.

In the use of canned sweet potatoes for culinary purposes it is sometimes desirable to have a product that is very firm and that will hold its form while being prepared for the table, as in the case of sautéing and candying. Some varieties which are attractive and satisfactory in other ways become soft when cooked, and are therefore unsuited for this purpose. A method of handling that would give this desired firmness in the product from the best table varieties would be of considerable advantage to the housewife.

In the earlier studies mentioned firmness or consistency tests were performed upon the product of all the varieties studied, which had been handled in the usual way, and like tests were made upon the product from a few of the varieties canned immediately after the potatoes were harvested. Among those of the first group a few were found which yielded a product of moderately firm consistency but one still too soft to be entirely satisfactory. The product from the freshly dug potatoes, on the other hand, was found to be very firm in all cases, and this fact seemed to suggest a possible solution of the problem.

It was for the purpose of investigating further this phase of sweet-potato utilization, and of making more complete the information available on the chemical constitution of the sweet potato and the transformations taking place within it under different conditions, that the work was pursued for another season. The results of this study are presented in the present paper.

The literature pertinent to this work has been reviewed already in the paper above mentioned, but reference will be made to it again

¹ Received for publication April 2, 1926; issued October, 1926.

² Reference is made by number (italic) to "Literature cited," p. 643.

from time to time in the present text wherever it will help to clarify the subject under discussion.

In all, 29 named varieties of sweet potatoes, together with about one-half dozen unnamed sorts were available for the work. These, arranged in alphabetical order, were: Ballinger's Pride, Big Stem Jersey, Catawba Yellow, Creola, Dahomey, Dooley, Florida, Georgia, Gold Skin, Gros Grandia, Haiti, Key West, Marneyita, Miles, Nancy Hall, Pierson, Porto Rico, Pumpkin "Yam," Purple "Yam," Red Bermuda, Red Brazil, Southern Queen, Triumph, Vineless Pumpkin "Yam," Vineless "Yam," White Sealy, Yellow Belmont, Yellow Jersey, and Yellow Strasburg. The unnamed sorts were identified by accession numbers only.

The potatoes were grown for the work at the Arlington Experiment Farm near Rosslyn, Va.

PLAN OF WORK

The plan of the experiments called for practical canning tests upon the potatoes immediately after they were dug and again after a month of storage at the end of the usual curing treatment,³ particular attention being given to the relative firmness of the canned product, its appearance and table quality, and its adaptability to various culinary uses as determined by practical kitchen tests. Samples for chemical analysis were taken of each variety, both raw and after cooking, at the time the canning tests were made, in order that the causes for change in the physical characteristics of the canned product as the result of curing and storage might be carefully studied. The record of these experiments follows.

EXPERIMENTAL METHODS

The digging of the crop was started October 17, 1922, and the work of canning and sampling for chemical analysis was begun the same day. One lot of each variety was canned immediately and the other was placed in the curing chamber. As soon as the different varieties were canned the curing of the second lot was begun. The curing treatment was continued until November 3. On this date the fire was extinguished and the temperature allowed to drop to 55° to 65° F., at which it was maintained throughout the storage period. On November 27 the canning and sampling for chemical analysis was begun again and the final work completed on December 2, 1922.

Uniform canning methods were followed throughout the experimental work, and the technic of chemical sampling and analysis was the same as that used in previous studies (10).

The quantity of raw stock used in the canning experiments varied somewhat with the different varieties, but averaged about 30 pounds for the freshly dug potatoes and 25 pounds for the cured and stored stock. Where larger yields in the field permitted, the size of the sample was increased, and in some instances where yields were light and the amount of material available was less, the size of the sample was smaller.

³ The curing treatment consisted in holding the potatoes in open slatted crates for 10 days in a curing chamber the temperature of which was maintained at about 85° F., with ample ventilation to allow the moisture driven off in the curing process to escape. At the end of this time the temperature was allowed to drop to 55° to 65° F. and the potatoes held under these conditions for one month.

Two types of canned product were packed: One, as whole potatoes, for which the No. 3 plain tin can was used; and the other, in the form of pulp or pie stock, for which No. 2 cans were employed.

Preparation for the can consisted in steaming the washed potatoes in a steam box until practically cooked (which required from 40 to 50 minutes) peeling at once by hand, packing hot into the cans, and sealing immediately. For the preparation of pie stock the hot freshly peeled potatoes were passed through the food grinder, placed immediately in the cans, and sealed.

TABLE 1.—*Peeling quality and percentage of waste of sweet potatoes canned fresh from the field and those canned after curing and storage*

Variety of sweet potato used	Sweet potatoes freshly dug			Sweet potatoes after curing and storage		
	Weight of sample in pounds	Peeling quality	Percentage waste	Weight of sample in pounds	Peeling quality	Percentage waste
Ballinger's Pride	40	Poor	12½	25	Poor to fair	17
Big Stem Jersey	30	Fair	13½	25	Poor	16
Catawba Yellow	30	do	16½	25	Fair to poor	18
Creola	8	do	18¾	7	Good	25
Dahomey	30	Poor	14	25	Poor	15
Dooley	30	Good	12½			
Florida	25	Fairly good	16	25	Poor	18
Georgia	30	do	14	25	Good	15
Gold Skin	30	do	11	25	do	16
Gros Grandia	8	Poor	18¾			
Haiti	30	do	11½	25	Fair	22
Key West	30	Very good	13½	25	do	23
Marneyita	12	Good	12½			
Miles	30	Fair	11½	25	Fair to poor	15
Nancy Hall	30	do	14	25	Good	15
Pierson	30	do	11	25	Poor	16
Porto Rico	60	do	13¾	25	Very good	16
Pumpkin "Yam"	30	Very good	15	25	Fair	15
Purple "Yam"	30	do	10	25	do	16
Red Bermuda	30	Poor	12½	25	do	14
Red Brazil	30	do	12½	20	do	16¾
Southern Queen	30	do	11	25	Good	14
Triumph	30	do	14	25	do	18
Vineless Pumpkin "Yam"	30	Good	11	16	do	17½
Vineless "Yam"	18	Fair	14	10	Fair	17½
White Sealy	9	Very good	11	25	Poor	20
Yellow Belmont	35	Good	13½			
Yellow Jersey	30	Fair	13½	25	Good	13
Yellow Strasburg	30	Good	11½	25	Poor	34
No. 10412	30	Poor	11½	25	do	21
No. 10650	30	do	11½	25	Fair	28
No. 11284	30	do	11½			
No. 12686	30	Fair	13½	25	Good	14
No. 24013	30	Poor	11½	12	do	14½
No. 24014	30	do	13½	25	do	17
No. 24015	35	do	13½	10		27½

The processing was performed as quickly as a sufficient number of cans could be packed to fill the retort. While awaiting the processing, all cans were kept submerged in a tank of water held at about 80° C. to prevent the contents from cooling. No. 3 cans were processed in the steam retort for 1 hour at 116° C., and the No. 2 cans for 45 minutes at the same temperature.

Records were kept of the weight of each sample, the peeling quality, and the amount of waste of each variety as it was handled.

At the end of the canning season samples of the different products from each of the varieties were opened for critical study, and their comparative qualities judged by a committee made up of canned-food experts, specialists in home economics, and others qualified by

experience to judge the merits of the products. Other series of samples from each of the varieties were subjected to plasticity tests to determine the relative firmness of the products, and products from selected representative varieties were turned over to the experimental kitchen of the Bureau of Chemistry for practical cooking tests.⁴

TESTS FOR PEELING QUALITY AND WASTE

Table 1, which records the findings made at the time the canning was done, tells its own story and calls for little comment. Owing to the fact that the bulk of the material used in each case was small, the figures can not with safety be used as an index of just what percentage loss would obtain when large-scale canning operations are carried on. When analyzed in connection with the previous work (10), however, they give a fair idea of what may be expected under commercial conditions.

It is seen that in the case of both the freshly dug stock and that which had been cured and stored, the peeling quality ranged from poor to very good. In the case of about a dozen different varieties the peeling quality was satisfactory under both conditions, while half that number peeled better in the freshly dug condition, and about a dozen better after the curing and storage.

The percentage of waste ranged from 10 to 18¾ per cent in the freshly dug stock and from 13 to 34 per cent in the cured and stored material. Variation in the amount of waste in the freshly dug potatoes was due primarily to the size of the potatoes; but in the cured stock some loss was sustained through discoloration caused by fungi which occasionally affected individual potatoes.

PLASTICITY TESTS

Of particular interest in the present study was the relative firmness or plasticity of the different products from the same varieties when canned fresh from the field as compared with those from cured and stored stocks. Plasticity tests, the results of which are given in Table 2, were made by observing the resistance to penetration of the canned material by a plunger having a cross-section of one-half square centimeter. The figures given represent the number of grams pressure required to force the plunger into the product to a distance of 2 cm. in 1 minute of time, and are averages from several tests made upon the material at both ends of the can.

The varieties are arranged in the order of greatest resistance in the material from freshly dug potatoes.

The outstanding fact revealed by these figures is the very great difference in plasticity of the product from the freshly dug potatoes as compared with that from the cured stock. The pressure required to force the plunger into the material in the first series ranged from 250 to 1,400 gm., whereas in the product from the cured potatoes only 20 to 180 gm. of pressure was required. In other words, the softest product from the freshly dug potatoes was considerably firmer than that of the driest material obtained after the storage treatment. In every instance there was a very marked reduction in the firmness as a result of the storage.

⁴ The writers are indebted to L. H. Bailey, of the Bureau of Chemistry, who superintended this part of the work, to Miss R. L. Rutledge, who made the cooking tests, and to F. C. Blanck, W. W. Skinner, P. L. Gowen, and H. C. Gore, also of the Bureau of Chemistry, who passed judgment upon the various sweet-potato dishes prepared from these products.

TABLE 2.—*Relative plasticity of the products from sweet potatoes of different varieties when canned fresh from the field and after curing and storage*

[The figures represent the pressure in grams required to force the plunger into the material to a distance of 2 cm. in 1 minute of time]

Variety of sweet potato used	Resistance of sweet potato when—		Variety of sweet potato used	Resistance of sweet potato when—	
	Freshly dug	Cured and stored		Freshly dug	Cured and stored
	Grams	Grams		Grams	Grams
Yellow Belmont.....	1,400	---	Marneyita.....	575	---
Florida.....	1,000	65	Nancy Hall.....	550	20
Purple "Yam".....	975	180	No. 24013.....	550	110
No. 24015.....	950	80	Yellow Strasburg.....	525	100
Gros Grandia.....	950	---	No. 12686.....	500	70
Vineless "Yam".....	950	50	Ballinger's Pride.....	500	40
No. 24014.....	875	80	Dooley.....	500	---
White Sealy.....	750	55	No. 10412.....	475	120
Pierson.....	725	100	No. 10650.....	450	75
Vineless Pumpkin "Yam".....	700	30	Gold Skin.....	425	80
Dahomey.....	675	180	Red Bermuda.....	425	80
Creola.....	650	25	Key West.....	375	70
No. 11284.....	625	---	Yellow Jersey.....	375	180
Haiti.....	625	85	Big Stem Jersey.....	350	120
Miles.....	600	40	Catawba Yellow.....	300	35
Pumpkin "Yam".....	600	40	Red Brazil.....	275	85
Triumph.....	600	85	Southern Queen.....	275	60
Georgia.....	575	45	Porto Rico.....	250	25

It is noted that there are relatively few varieties showing any marked degree of firmness in the product from the stored potatoes. Two of these, Dahomey and Purple "Yam," because of their elongated rootlike form, rather poor peeling quality, and tendency to discolor in the can are hardly suited for general canning purposes. The two Jersey potatoes, Big Stem Jersey and Yellow Jersey, because of their favorable shape and size and their desirable table qualities are used more extensively for canning than any other of the drier sorts. It is seen, however, that where firmness combined with high color is desired in the finished product a wide range of varieties is available if the potatoes are canned when freshly dug.⁵

That a better idea may be gained of the difference in firmness or plasticity of the sweet-potato product when it is canned fresh from the field and after it has been cured and stored for one month, Figures 1 to 4, inclusive, are shown. The variety used in the illustrations was Nancy Hall, well known as one of the best soft cooking varieties. Figure 1 shows the product from the freshly dug potatoes canned whole. Figure 2 shows the same, canned after the curing and storage treatment. Figures 3 and 4 illustrate the product canned as pie stock under the same conditions as in Figures 1 and 2.

Little comment on these illustrations is required. Attention is called to the firmness of the products shown in Figures 1 and 3 as compared with the softness and moistness of the products illustrated in Figures 2 and 4.

It will be of interest at this point to consider the chemical composition of the different varieties of sweet potatoes in relation to the difference in plasticity, as the changes in physical characteristics are due to alterations in the chemical constitution of the potatoes as a result of the curing and storage treatment.

⁵ Those interested in the color factor in the canned product of sweet potatoes will find a series of color plates showing the range of colors found among the different varieties, in the U. S. Department of Agriculture Bulletin No. 1041 entitled, "A Study of Sweet-Potato Varieties, with Special Reference to Their Canning Quality" (10).

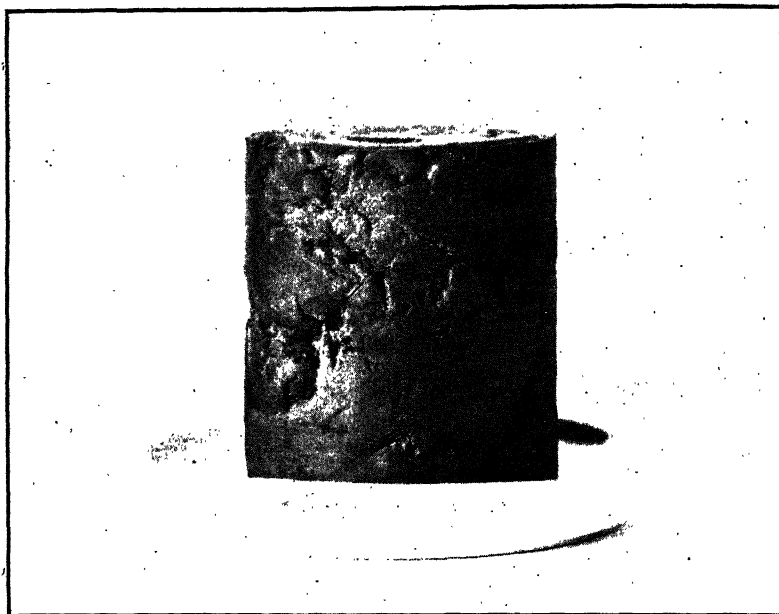


FIG. 1.—Product of the Nancy Hall sweet potato canned whole immediately after digging

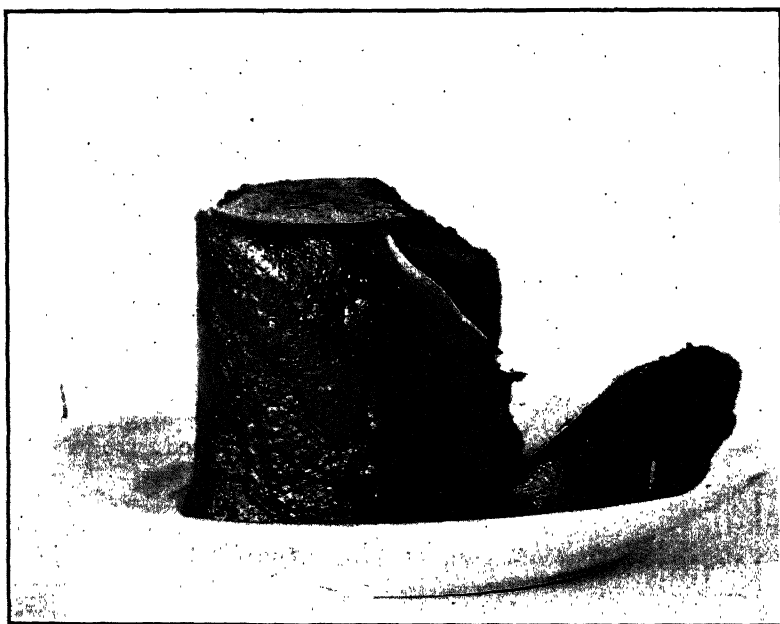


FIG. 2.—Product of the Nancy Hall sweet potato canned whole after the curing and storage treatment

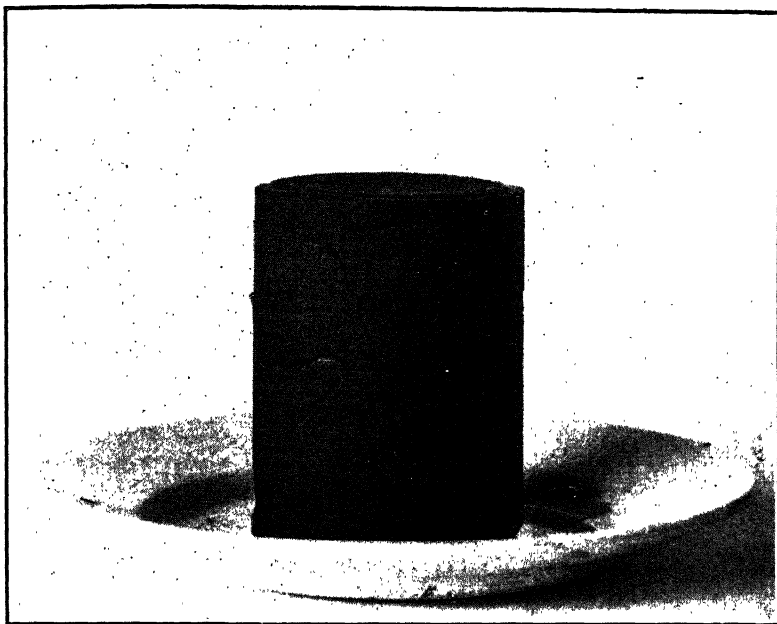


FIG. 3.—Product of the Nancy Hall sweet potato canned as pie stock immediately after digging

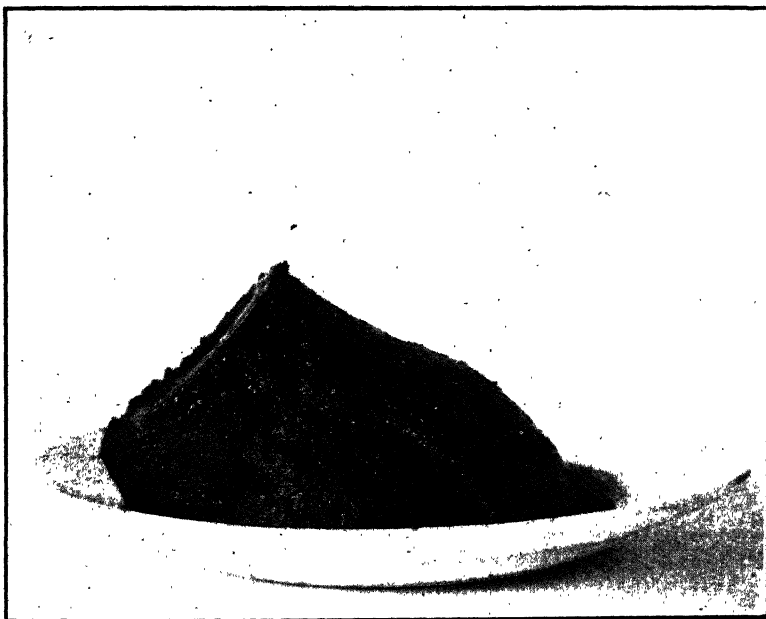


FIG. 4.—Product of the Nancy Hall sweet potato canned as pie stock after the curing and storage treatment

CHEMICAL STUDIES

These studies are a continuation and extension of chemical work previously reported by the writers (10, 11), and is here made to include all the important varieties of sweet potatoes grown in this country at the present time. The methods of analysis were essentially the same as reported in the earlier work. Samples were taken from a number of representative potatoes both raw and cooked, at the time of digging and after curing and storage.

To 100 gm. of the sample enough 95 per cent alcohol was added to make the alcohol concentration 75 to 80 per cent. After extraction with alcohol the extract was made up to volume and the sugars determined in the usual way by the copper reduction method. The sucrose was inverted by boiling with 2 per cent citric acid, as recommended by Davis and Daish (2), in order to avoid inversion of maltose. The total polysaccharides were determined⁶ by inversion with hydrochloric acid as recommended by the Association of Official Agricultural Chemists (1). The dextrin was estimated as described by the writers (11), except that the tedious filtrations were avoided by centrifuging at high speed in a large centrifuge of standard make.

The reducing sugars were calculated in two ways: In the first, the total reducing sugars both before and after cooking of the potatoes were calculated as invert sugar; in the second, the increase after cooking was calculated as maltose. In making this estimate it was assumed that all the sugar formed during cooking was maltose. This method of estimating maltose is, of course, indirect and is subject to the error of random sampling. Owing to the varying amounts of the different sugars in such a complex mixture the method may be subject to considerable error. In most cases the estimated maltose very nearly accounted for the decrease in starch during cooking.

The results of this work are presented in Table 3.

MOISTURE CONTENT

Table 3 shows that the samples vary considerably in their percentage of dry matter. Johnstone (8) found characteristic differences in sweet potatoes of the Porto Rico and Triumph varieties. Harrington (5) investigated the moisture content of sweet potatoes during storage and found a decrease from 71.34 to 63.5 per cent after several months of storage. His results showed that there was a considerable variation in the moisture content of potatoes of different varieties. Shiver (13) found that the moisture content was variable during storage, and the results of many other investigators indicate that the moisture varies with the seasonal conditions and with the methods of storage after digging.

The present results show a variation in the moisture content of from 27 to 39 per cent. In almost any lot individual potatoes of any variety will vary from 4 to 6 per cent in moisture. Numerous determinations have shown that in general the larger the potatoes the higher the moisture content. However, the age of the potatoes is a factor, as has been shown by Keitt (9). He found that the young

⁶ The determinations of total polysaccharides were made by B. C. Brunstetter.

potato had a high solid content followed by a period of rapid growth in which the solid content was low. As the potato approached maturity the growth was slower while starch was being laid down, resulting in a higher solid content in the mature roots.

Because of these variations in moisture content the sampling error may sometimes be considerable in spite of efforts to avoid it. In the present results there are a few cases in which the moisture of the raw and the cooked potatoes varied from 1 to 2 per cent. However, the differences are usually less than 1 per cent. In most cases where there are differences shown the raw sample is higher, though in several cases the reverse is true. Only a small amount of evaporation was possible in the method of handling used and the differences shown are mainly due to sampling error.

Generally, the moisture content found is characteristic of the variety, with the possible error of 2 or 3 per cent. Triumph, Georgia, and Purple "Yam" are characterized by a very high solids content. Numerous moisture determinations on these during the last four years have shown a solids content varying from 35 to 40 per cent, with an average of about 37 per cent. Gold Skin has the lowest solids content of any variety, and though this result is somewhat below the average for several years, it is always strikingly low in solids. This probably explains why potatoes of this variety are likely to shrivel and shrink severely under certain storage conditions. The Jersey group as a whole is below the average in solids. Key West and Creola are also low in solids, which partly accounts for their consistency when cooked. Porto Rico is also frequently rather low although the determination in this case is somewhat below the average for four years. Potatoes of a larger number of varieties are quite similar in their solids content and average from 32 to 35 per cent.

The quantity of moisture present is an important factor in causing the difference in quality of varieties by modifying the firmness or softness of the canned product. The very high solids content makes the sweet potato a very concentrated food.

SUGARS

Miyake (12) identified dextrose, levulose, and sucrose in the sweet potato. He obtained no evidence, however, of the presence of pentose, galactose, mannose, or maltose. A number of workers (5, 6, 7, 9, 10, 11, 12, 13, 14) have reported the presence of both reducing and nonreducing sugars in the sweet potato. The writers (10) found large quantities of reducing sugars in the cooked potatoes, and later showed (11) that large quantities of these sugars, principally maltose, were formed during cooking.

Gore (4) found that practically all the sugar formed during the cooking of sweet potatoes was maltose. He also found (3) that the sweet potato was rich in diastase, and that only a small quantity of malt was necessary to convert all the starch into sugar. Hasselbring and Hawkins (6, 7) found that the sugar content of the sweet potato changed after digging and varied with the storage temperature. At low temperatures the sucrose increased and the reducing sugars decreased, which led them to the conclusion that starch was first changed to reducing sugars and that sucrose was then synthesized from these.

TABLE 3.—Results of chemical analysis of sweet potatoes of different varieties

Variety of sweet potato	Condition	Analysis made immediately after digging (per cent)										Analysis made after curing and storage (per cent)												
		Solids			Sugars				Polysaccharides			Solids			Sugars				Polysaccharides					
		Alcoholic extract	Residue	Total	Calculated as invert			Increase after cooking, calculated as maltose			Dextrin	Total	Alcoholic extract	Residue	Calculated as invert			Increase after cooking, calculated as maltose			Dextrin	Total		
					Total	Reducing	Sucrose	Total	Reducing	Sucrose					Total	Reducing	Sucrose	Total	Reducing	Sucrose			Maltose	
Ballinger's Pride.....	Raw.....	31.22	3.22	28.00	2.04	0.31	1.73	0.31	1.42	1.38	22.80	33.36	7.79	25.57	0.23	5.62	0.23	20.45	5.50	18.34	0.23	5.50	10.45	1.82
.....	Cooked.....	32.62	4.20	28.42	2.98	0.80	2.18	0.48	1.70	1.88	10.43	0.08	32.82	19.94	12.88	13.02	8.12	5.50	12.01	10.50	1.82	5.50	12.01	10.50
Big Stem Jersey.....	Raw.....	32.26	4.52	27.74	2.96	0.48	2.48	0.48	2.00	2.19	21.59	29.08	7.38	22.30	6.14	5.31	0.83	17.84	5.31	11.97	1.24	5.31	11.97	1.24
.....	Cooked.....	32.55	4.26	28.29	2.98	0.45	2.53	0.45	2.08	2.23	11.35	51.20	9.71	15.00	14.97	10.02	5.27	5.84	11.97	1.24	5.31	11.97	1.24	
Catawba Yellow.....	Raw.....	34.92	4.24	30.68	2.05	0.78	1.27	0.78	0.49	1.24	24.84	33.28	7.62	25.66	6.17	5.61	0.56	20.52	5.61	15.00	1.09	5.61	15.00	1.09
.....	Cooked.....	34.92	4.24	30.68	2.05	0.78	1.27	0.78	0.49	1.24	24.84	33.28	7.62	25.66	6.17	5.61	0.56	20.52	5.61	15.00	1.09	5.61	15.00	1.09
Creola.....	Raw.....	29.11	3.12	25.99	1.41	0.61	1.80	0.61	1.19	1.13	12.72	31.16	8.38	22.78	6.45	1.07	5.38	18.22	5.38	12.63	1.33	5.38	12.63	1.33
.....	Cooked.....	30.26	4.40	25.85	1.16	0.76	1.40	0.83	0.57	1.40	8.82	22.68	7.60	28.76	6.22	1.37	4.85	16.76	1.07	5.73	9.96	1.37	4.85	16.76
Dahomey.....	Raw.....	33.11	4.20	28.91	2.88	0.52	2.36	0.52	1.84	2.30	9.34	35.85	8.13	27.72	5.87	1.39	4.48	13.61	1.37	4.46	7.78	1.39	4.48	13.61
.....	Cooked.....	33.00	3.89	29.11	1.91	0.24	2.64	0.24	2.40	2.52	12.32	36.36	7.60	28.76	6.22	1.37	4.85	16.76	1.07	5.73	9.96	1.37	4.85	16.76
Dooley.....	Raw.....	32.73	3.32	29.41	1.08	0.18	1.50	0.18	1.32	1.64	11.14	35.85	8.13	27.72	5.87	1.39	4.48	13.61	1.37	4.46	7.78	1.39	4.48	13.61
.....	Cooked.....	34.81	4.62	30.19	2.20	0.76	1.44	0.76	0.68	1.12	25.12	36.36	7.60	28.76	6.22	1.37	4.85	16.76	1.07	5.73	9.96	1.37	4.85	16.76
Florida.....	Raw.....	35.81	3.18	32.63	1.85	0.15	1.70	0.15	1.55	1.64	11.14	35.85	8.13	27.72	5.87	1.39	4.48	13.61	1.37	4.46	7.78	1.39	4.48	13.61
.....	Cooked.....	36.18	1.61	34.57	0.91	0.70	2.21	0.70	1.51	2.21	14.02	36.36	7.60	28.76	6.22	1.37	4.85	16.76	1.07	5.73	9.96	1.37	4.85	16.76
Georgia.....	Raw.....	38.28	3.98	34.30	1.91	0.21	1.75	0.21	1.54	1.57	12.90	37.27	9.58	27.69	7.81	0.90	6.89	11.01	0.90	6.89	11.01	0.90	6.89	11.01
.....	Cooked.....	37.83	1.52	36.31	0.99	0.72	1.62	0.72	0.98	1.57	12.90	37.27	9.58	27.69	7.81	0.90	6.89	11.01	0.90	6.89	11.01	0.90	6.89	11.01
Gold Skin.....	Raw.....	28.89	5.30	23.59	4.03	0.68	3.34	0.68	2.66	3.24	10.40	28.90	6.81	22.09	5.09	1.95	6.14	10.50	1.95	6.14	10.50	1.95	6.14	10.50
.....	Cooked.....	27.87	4.81	23.06	3.82	0.76	3.06	0.76	2.30	3.24	10.40	28.90	6.81	22.09	5.09	1.95	6.14	10.50	1.95	6.14	10.50	1.95	6.14	10.50
Haiti.....	Raw.....	34.01	4.32	29.69	2.89	0.73	2.16	0.73	1.43	2.16	14.11	33.65	6.64	27.01	5.23	0.94	4.29	10.50	0.94	4.29	10.50	0.94	4.29	10.50
.....	Cooked.....	34.62	4.05	30.57	2.74	0.73	2.01	0.73	1.28	2.01	14.11	33.65	6.64	27.01	5.23	0.94	4.29	10.50	0.94	4.29	10.50	0.94	4.29	10.50
Key West.....	Raw.....	30.89	2.41	28.48	1.67	0.31	1.36	0.31	1.05	1.32	9.16	37.33	4.16	33.17	13.18	8.56	4.60	16.75	1.14	4.60	16.75	1.14	4.60	16.75
.....	Cooked.....	30.89	2.41	28.48	1.67	0.31	1.36	0.31	1.05	1.32	9.16	37.33	4.16	33.17	13.18	8.56	4.60	16.75	1.14	4.60	16.75	1.14	4.60	16.75
Marneyita.....	Raw.....	31.30	4.49	26.81	2.79	0.37	2.42	0.37	2.05	2.10	11.29	31.30	7.14	24.16	13.18	8.56	4.60	16.75	1.14	4.60	16.75	1.14	4.60	16.75
.....	Cooked.....	32.47	3.92	28.55	2.55	0.35	2.20	0.35	1.85	2.10	11.29	31.30	7.14	24.16	13.18	8.56	4.60	16.75	1.14	4.60	16.75	1.14	4.60	16.75
Miles.....	Raw.....	32.54	4.63	27.91	1.60	0.01	1.61	0.01	1.60	1.61	15.32	31.30	7.14	24.16	13.18	8.56	4.60	16.75	1.14	4.60	16.75	1.14	4.60	16.75
.....	Cooked.....	32.54	4.63	27.91	1.60	0.01	1.61	0.01	1.60	1.61	15.32	31.30	7.14	24.16	13.18	8.56	4.60	16.75	1.14	4.60	16.75	1.14	4.60	16.75
Nancy Hall.....	Raw.....	30.56	3.66	26.90	1.89	0.17	1.72	0.17	1.55	1.67	13.00	33.35	9.22	24.13	7.96	0.79	7.17	19.96	0.79	7.17	19.96	0.79	7.17	19.96
.....	Cooked.....	30.57	3.66	26.91	1.89	0.17	1.72	0.17	1.55	1.67	13.00	33.35	9.22	24.13	7.96	0.79	7.17	19.96	0.79	7.17	19.96	0.79	7.17	19.96

Pierson.	Raw	35.00	3.54	31.46	2.59	46	2.13	1.08	46	2.06	10.56	14.26	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
Porto Rico.	Cooked	30.16	4.07	25.09	5.06	7.60	2.44	1.26	8.85	2.63	12.82	10.26	31.34	8.85	24.49	11.56	7.96	3.79	1.43	3.97	92.21
Pumpkin "Yam"	Raw	31.94	3.18	28.76	3.18	8.41	2.03	1.30	8.41	2.03	12.82	10.26	31.34	8.85	24.49	11.56	7.96	3.79	1.43	3.97	92.21
	Cooked	34.04	3.16	30.88	3.16	7.16	2.00	1.30	7.16	2.00	12.82	10.26	31.34	8.85	24.49	11.56	7.96	3.79	1.43	3.97	92.21
Purple "Yam"	Raw	35.02	3.24	31.78	3.24	7.53	1.63	1.01	7.53	1.63	9.18	14.06	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
	Cooked	35.73	3.22	32.51	3.22	7.53	1.63	1.01	7.53	1.63	9.18	14.06	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
Red Bermuda.	Raw	35.17	4.29	30.88	4.29	8.27	2.70	13.76	18	1.70	11.79	16.50	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
	Cooked	35.50	4.57	30.93	4.57	7.48	2.09	12.69	52	1.97	10.20	12.84	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
Red Brazil.	Raw	32.76	4.16	28.60	4.16	7.48	2.09	12.69	52	1.97	10.20	12.84	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
	Cooked	34.98	4.17	29.81	4.17	8.66	2.07	14.65	58	2.07	12.00	13.14	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
Southern Queen.	Raw	33.30	3.96	29.34	3.96	7.01	2.08	12.09	51	2.08	9.50	12.05	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
	Cooked	31.00	5.28	26.72	5.28	7.01	2.08	12.09	51	2.08	9.50	12.05	34.77	7.00	27.77	4.94	3.51	14.96	1.43	3.97	92.21
Triumph.	Raw	38.28	3.98	34.30	3.98	28	2.10	1.16	28	2.10	1.16	13	38.28	3.98	34.30	3.98	28	2.10	1.16	13	38.28
	Cooked	38.29	4.04	34.25	4.04	9.48	2.22	13.18	38	2.22	10.58	14.34	38.29	4.04	34.25	4.04	9.48	2.22	13.18	38	2.22
Vineless Pumpkin "Yam"	Raw	33.94	4.62	29.32	4.62	7.56	2.47	1.81	7.56	2.47	1.81	1.81	33.94	4.62	29.32	4.62	7.56	2.47	1.81	1.81	33.94
	Cooked	34.39	4.62	29.32	4.62	7.56	2.47	1.81	7.56	2.47	1.81	1.81	33.94	4.62	29.32	4.62	7.56	2.47	1.81	1.81	33.94
Vineless "Yam"	Raw	35.78	5.50	32.28	5.50	7.31	1.72	12.35	17	1.72	10.46	17.91	35.78	5.50	32.28	5.50	7.31	1.72	12.35	17	1.72
	Cooked	36.42	5.01	32.41	5.01	7.31	1.72	12.35	17	1.72	10.46	17.91	35.78	5.50	32.28	5.50	7.31	1.72	12.35	17	1.72
White Sealy.	Raw	32.09	3.94	28.15	3.94	4.46	1.95	1.30	4.46	1.95	1.30	1.30	32.09	3.94	28.15	3.94	4.46	1.95	1.30	1.30	32.09
	Cooked	32.01	4.04	27.97	4.04	7.11	1.81	10.30	46	1.81	8.03	14.37	32.01	4.04	27.97	4.04	7.11	1.81	10.30	46	1.81
Yellow Jersey.	Raw	28.17	5.02	24.15	5.02	6.11	2.53	1.77	6.11	2.53	1.77	1.77	28.17	5.02	24.15	5.02	6.11	2.53	1.77	1.77	28.17
	Cooked	30.32	3.86	26.46	3.86	5.95	2.92	10.77	61	2.92	7.24	13.42	30.32	3.86	26.46	3.86	5.95	2.92	10.77	61	2.92
Yellow Strasburg.	Raw	33.39	3.96	29.43	3.96	7.78	1.88	1.72	7.78	1.88	1.72	1.72	33.39	3.96	29.43	3.96	7.78	1.88	1.72	1.72	33.39
	Cooked	34.01	4.72	29.29	4.72	7.78	1.88	1.72	7.78	1.88	1.72	1.72	34.01	4.72	29.29	4.72	7.78	1.88	1.72	1.72	34.01
No. 10412.	Raw	32.62	4.28	28.34	4.28	4.86	1.88	1.40	4.86	1.88	1.40	1.40	32.62	4.28	28.34	4.28	4.86	1.88	1.40	1.40	32.62
	Cooked	34.21	3.25	31.96	3.25	8.46	2.18	14.60	48	2.18	11.94	12.21	34.21	3.25	31.96	3.25	8.46	2.18	14.60	48	2.18
No. 12686.	Raw	34.80	4.47	30.33	4.47	8.63	2.13	13.75	98	2.13	10.84	13.39	34.80	4.47	30.33	4.47	8.63	2.13	13.75	98	2.13
	Cooked	34.82	4.43	30.30	4.43	8.25	1.95	13.75	98	1.95	10.84	13.39	34.82	4.43	30.30	4.43	8.25	1.95	13.75	98	1.95
No. 24013.	Raw	34.92	3.43	31.49	3.43	6.96	1.91	12.27	15	1.91	10.21	21.76	34.92	3.43	31.49	3.43	6.96	1.91	12.27	15	1.91
	Cooked	34.92	3.43	31.49	3.43	6.96	1.91	12.27	15	1.91	10.21	21.76	34.92	3.43	31.49	3.43	6.96	1.91	12.27	15	1.91
No. 24014.	Raw	30.96	3.70	27.26	3.70	1.85	2.98	1.62	1.85	2.98	1.62	1.62	30.96	3.70	27.26	3.70	1.85	2.98	1.62	1.62	30.96
	Cooked	33.90	4.60	29.30	4.60	6.97	1.85	11.62	26	1.85	9.49	15.44	33.90	4.60	29.30	4.60	6.97	1.85	11.62	26	1.85
No. 24015.	Raw	33.91	3.86	30.07	3.86	7.71	1.87	1.39	7.71	1.87	1.39	1.39	33.91	3.86	30.07	3.86	7.71	1.87	1.39	1.39	33.91
	Cooked	33.91	3.86	30.07	3.86	7.71	1.87	1.39	7.71	1.87	1.39	1.39	33.91	3.86	30.07	3.86	7.71	1.87	1.39	1.39	33.91

1 Trace.

REDUCING SUGARS

Table 3 shows that the reducing sugars in the uncooked potatoes were always small in amount and quite variable in different samples, even in the same variety. Since the quantity present is nearly always less than 1 per cent these variations are not large in total amount and are of no particular significance in canning operations. In most cases there is an increase in the amount present during the curing and storage period. There are some cases, however, where the reverse is true.

MALTOSE

After cooking there is always a very great increase in the reducing sugars. If this increase is calculated as maltose there appears to be 7 to 14 per cent present in the different varieties. This increase is accompanied by a corresponding decrease in starch. This indicates that there is a large amount of diastase present which varies considerably in the different varieties. Since maltose arises from the conversion of starch this change greatly affects the softness of the canned product. However, the softness is not proportional to the amount of maltose present, especially in the different varieties. Inconsistencies such as this lead one to conclude that there may be other very important factors that are also unexplained.

Among those varieties high in maltose are Triumph, Nancy Hall, Porto Rico, Yellow Strasburg, Purple "Yam," Georgia, Miles, and Marneyita. Generally there is at least a small increase in the maltose in the cooked samples after curing and storage, though in some cases the reverse is true, which may be due merely to sampling error. The large quantity of maltose present greatly affects the quality of the product by its added sweetness; but because of its lesser sweetening power it is not so important as sucrose.

SUCROSE

Sucrose is the most abundant sugar in the raw material and the variation in the amount of it present greatly affects the sweetness of the canned material. There seems to be practically no change in it during cooking. Such variations as occur in the analyses seem to have resulted from errors in sampling. An increase in sucrose always occurs during curing and storage. There is from three to four times as much present in the cured as in the freshly dug samples. This tremendously affects the sweetness and the quality of the canned material and is the chief factor giving higher quality to the cured and stored material. There is not a very great variation in the amount of sucrose in potatoes of different varieties when freshly dug, but the variations are greater in the cured material, ranging from 3.5 to 7.5 per cent. Nancy Hall, Pumpkin "Yam," Vineless Pumpkin "Yam," Vineless "Yam," Georgia, Yellow Strasburg, and Porto Rico are particularly high in sucrose in the cured and stored material.

POLYSACCHARIDES

STARCH

The hydrolyzable polysaccharides of the raw sweet potato consist almost entirely of starch. The amount present differs considerably with the varieties, stage of growth, and storage conditions. In the raw state the percentage of starch present is correlated quite closely

with the moisture content, being high when the moisture is low. It decreases during storage as a result of its conversion into sucrose. During cooking large quantities are converted into soluble products, chiefly maltose, but in some varieties considerable quantities are changed to dextrinlike forms. Starch is the chief constituent giving firmness to the canned product. In most varieties there is more or less starch remaining which, during the cooking process, is modified only by being gelatinized. The consistency of the canned material is very nearly proportional to the ratio of starch to moisture. Some of the raw samples are high in starch, namely, Triumph, Florida, Catawba Yellow, Nancy Hall, Vineless "Yam," and, immediately after digging, Pumpkin "Yam."

DEXTRIN

Stone (14) very early found dextrin present in cooked sweet potatoes and showed that it was formed during cooking. The writers (10), working with four varieties, found comparatively wide variations in the dextrin content and showed that there was close correlation between the amount of dextrin present and the softness of the cooked product. The dextrin arises from the partial conversion of starch during cooking probably as the result of the activity of diastase or other enzymes.

By comparing the figures in Table 2 showing relative consistency with those for dextrin in Table 3, it will be noted that in every case where there is a high dextrin content the material is soft in consistency. High dextrin is nearly always associated with high maltose content, but in many cases there is a high maltose content unaccompanied by high dextrin content. Dextrin does not seem to affect the quality except as it does so through modifying the consistency.

QUALITY OF PRODUCTS

It is hardly possible to convey by the usual terms just what is meant by "good" or "poor" canning quality when speaking of freshly dug sweet potatoes. High quality in sweet potatoes is, as already noted, usually associated with high sugar content, pleasing flavor, attractive color, and proper consistency. In the product from the freshly dug potatoes the sucrose content is relatively low, the flavor is less well developed, and the consistency always firm. Quality in this case applies more particularly to attractiveness of the product as determined by color and brightness; somewhat less to flavor; but particularly to its suitability for certain culinary uses for which the cured potato, because of its soft consistency, is not adapted. This product would supplement rather than supplant the usual canned sweet potato.

In the present studies both the freshly dug and the cured potatoes were canned and the product of each subjected to careful judging as to quality. With respect to the product from the cured potatoes the findings were in all cases practically identical with those previously obtained, which have been set forth in more or less detail by the writers in the publication previously mentioned (10). There it is recorded that Gold Skin was given first place, and Porto Rico, Nancy Hall, and Vineless Pumpkin "Yam" of the moist fleshed group, and Big Stem Jersey, Improved Big Stem, Yellow Strasburg, and

Triumph of the firmer fleshed varieties were accorded high ratings in about the order given.

Observations upon the canning qualities of the freshly dug sweet potatoes of the different varieties in the present study are given briefly in Table 4.

TABLE 4.—Qualities of the canned product from freshly dug sweet potatoes

Variety of sweet potato used	Attractiveness	Consistency	Tendency to darken on exposure	Table quality
Ballinger's Pride.....	Fair to good.....	Firm.....	Slight.....	Poor.
Big Stem Jersey.....	do.....	Fairly firm.....	Little.....	Medium to poor.
Creola.....	do.....	Firm.....	do.....	Fair.
Dahomey.....	Poor.....	Very firm.....	Pronounced.....	Poor.
Dooley.....	Fair.....	Firm.....	Little.....	Fair.
Florida.....	do.....	Very firm.....	do.....	Poor.
Georgia.....	Poor.....	Firm.....	Pronounced.....	Do.
Gold Skin.....	Very good.....	Fairly firm.....	Very little.....	Good.
Gros Grandia.....	Poor.....	Very firm.....	Pronounced.....	Poor.
Haiti.....	Fair.....	Firm.....	Little.....	Fair.
Key West.....	Rather poor.....	Fairly firm.....	do.....	Poor.
Marneyita.....	Fair.....	Firm.....	do.....	Rather poor.
Miles.....	do.....	do.....	Pronounced.....	Poor.
Nancy Hall.....	Very good.....	do.....	Little.....	Very good.
Pierson.....	Fair.....	Very firm.....	do.....	Very poor.
Porto Rico.....	Very good.....	Fairly firm.....	do.....	Fairly good.
Pumpkin "Yam".....	Fair.....	Firm.....	Pronounced.....	Fair.
Purple "Yam".....	Poor.....	Very firm.....	do.....	Poor.
Red Bermuda.....	Fair to good.....	Firm.....	Little.....	Do.
Red Brazil.....	do.....	Fairly firm.....	do.....	Do.
Southern Queen.....	Rather poor.....	do.....	Pronounced.....	Rather poor.
Triumph.....	Good.....	Firm.....	Little.....	Poor.
Vineless Pumpkin "Yam".....	Very good.....	do.....	do.....	Very good.
Vineless "Yam".....	Fair.....	do.....	do.....	Poor.
White Sealy.....	do.....	do.....	do.....	Fair.
Yellow Belmont.....	Rather poor.....	Very firm.....	Pronounced.....	Poor.
Yellow Jersey.....	do.....	Fairly firm.....	Little.....	Do.
Yellow Strasburg.....	Fair to good.....	Firm.....	Pronounced.....	Fair.
No. 10412.....	Poor.....	do.....	do.....	Very poor.
No. 10650.....	Good.....	do.....	Little.....	Poor.
No. 11284.....	do.....	do.....	do.....	Fair.
No. 12686.....	Fair.....	do.....	do.....	Fair to good.
No. 24012.....	Fair to good.....	do.....	do.....	Poor.
No. 24013.....	Poor.....	do.....	do.....	Do.
No. 24014.....	Good.....	Very firm.....	do.....	Do.
No. 24015.....	Very poor.....	do.....	Pronounced.....	Do.

It is seen that of these 36 different varieties and strains of sweet potatoes approximately 70 per cent graded from fair to very good from the standpoint of attractiveness of the canned product; all were firm in consistency; about 70 per cent showed little tendency to discolor when exposed to the air after being removed from the can; and about 30 per cent of them graded from "fair" to "very good" with respect to table quality. Although attractiveness, tendency to remain bright on exposure to the air, and good table quality were not always found in those varieties showing one or more of these qualities, it is of interest to note that a considerable number of them did combine all of these qualities; as, for instance, Gold Skin, Nancy Hall, Porto Rico, and Vineless Pumpkin "Yam."

In this connection the results of the judging by the committee of canned-food specialists will be of interest. Although there was not complete agreement in all cases among individual judges as to the merits of the canned product of the different varieties, Nancy Hall was given first place by a large majority of the judges, with Gold Skin ranking second. With some, Vineless Pumpkin "Yam" was considered as second only to Nancy Hall, and favorable mention was made of Porto Rico, Big Stem Jersey, Triumph, Red Bermuda, and several others.

COOKING TESTS

To determine the suitability for culinary uses of the canned product from freshly dug sweet potatoes as compared with that from cured potatoes, a number of samples of selected varieties were submitted to the experimental kitchen of the Bureau of Chemistry for practical cooking tests, and the results were judged by a committee of food experts from that bureau. The samples submitted included both the whole and the pulped or pie-stock canned sweet potatoes of the freshly dug and the cured and stored Vineless Pumpkin "Yam," Gold Skin, and Triumph varieties, and the whole potato canned material of the Porto Rico. These were opened on the morning of January 5, 1923, and the following products and dishes prepared: (1) Pie fillers from the pie stock material of both the freshly dug and the cured and stored potatoes; (2) pies with crusts, from the freshly dug potato stock; (3) sliced and sautéed sweet potatoes from the freshly dug potato product (the cured potato product was too soft for slicing); (4) sliced and candied pie stock from the freshly dug potato product, except in the case of the Porto Rico, in which whole potato canned product was used.

For the preparation of pie fillers and pies it was found necessary to pass the canned material through a potato ricer because of the firmness of the product.

The results of these practical cooking tests are briefly described in the following summaries:

VINELESS PUMPKIN "YAM"

Pie filler.—Somewhat grained in texture but excellent in appearance and flavor. Filler prepared from the cured potato stock was smoother in texture.

Pies.—Rather coarse grained owing to the curdling of the milk during preparation and cooking. In all other respects the pies were satisfactory.

Sautéed potatoes.—Good, the slices remaining intact during the cooking and the product being very attractive and satisfactory.

Candied sweet potato.—The slices crumbled somewhat, but otherwise the product was excellent.

GOLD SKIN

Pie filler.—Very slightly grained but excellent in appearance and flavor. This filler would be very acceptable anywhere. Filler from cured potato stock was a little smoother in texture.

Pies.—Very good, somewhat smoother in texture than those from the Vineless Pumpkin "Yam."

Sautéed potatoes.—Very fine product, the slices remaining intact during the cooking. Appearance and flavor very satisfactory.

Candied sweet potato.—Excellent. Slices held their form perfectly and made a most pleasing and attractive dish.

TRIUMPH

Pie filler.—Good. Slightly grained in texture but not enough to be objectionable. The filler from the cured potato stock was a little smoother in texture.

Pies.—Good. Slightly grained in texture.

Sautéed potatoes.—Excellent. Slices held their form perfectly.

Candied sweet potato.—Very good. Slices held their form in the preparation and made an attractive dish.

PORTO RICO

Pie filler.—Good, but slightly grained in texture and showed some fibers which were somewhat objectionable. Filler from the cured potato stock was a little smoother in texture but the fibers were too much in evidence for the best results.

Pies.—Very satisfactory, though slightly grained.

Sautéed potatoes.—Satisfactory. Slices held their form perfectly although made from whole potato canned product.

Candied sweet potato.—A very attractive dish. Slices held their form perfectly.

In judging the merits of the different varieties in these cooking experiments the committee was nearly unanimous in giving first place to Gold Skin, with Vineless Pumpkin "Yam" and Porto Rico tying for second place.

From these studies it is seen that in the canned product from the freshly dug sweet potatoes the housewife has an excellent material for a number of attractive dishes, some of which can not be prepared satisfactorily from the sweet potatoes canned after curing and storage.

SUMMARY

The study herein reported has shown that during curing and storage profound transformations take place in the sweet potato, and further alterations occur during the cooking process.

The physical evidence of these transformations, as they are manifested in the canned product, are a very marked softening of the flesh of the potato and a very considerable increase in sweetness. In all of the varieties studied the freshly dug potatoes yielded a very firm product, whereas the cured and stored stock yielded a product varying all the way from a medium to a very soft consistency.

The chemical changes taking place are evidenced by a transformation of part of the starch to dextrin and sucrose, thus altering the starch moisture ratio, and during the cooking, a splitting of a portion of the starch to maltose. These changes are responsible for the altered physical consistency of the canned product and its increase in sweetness. The ratio of starch to moisture determines the consistency, and the chief factor causing the difference in sweetness is the amount of sucrose formed.

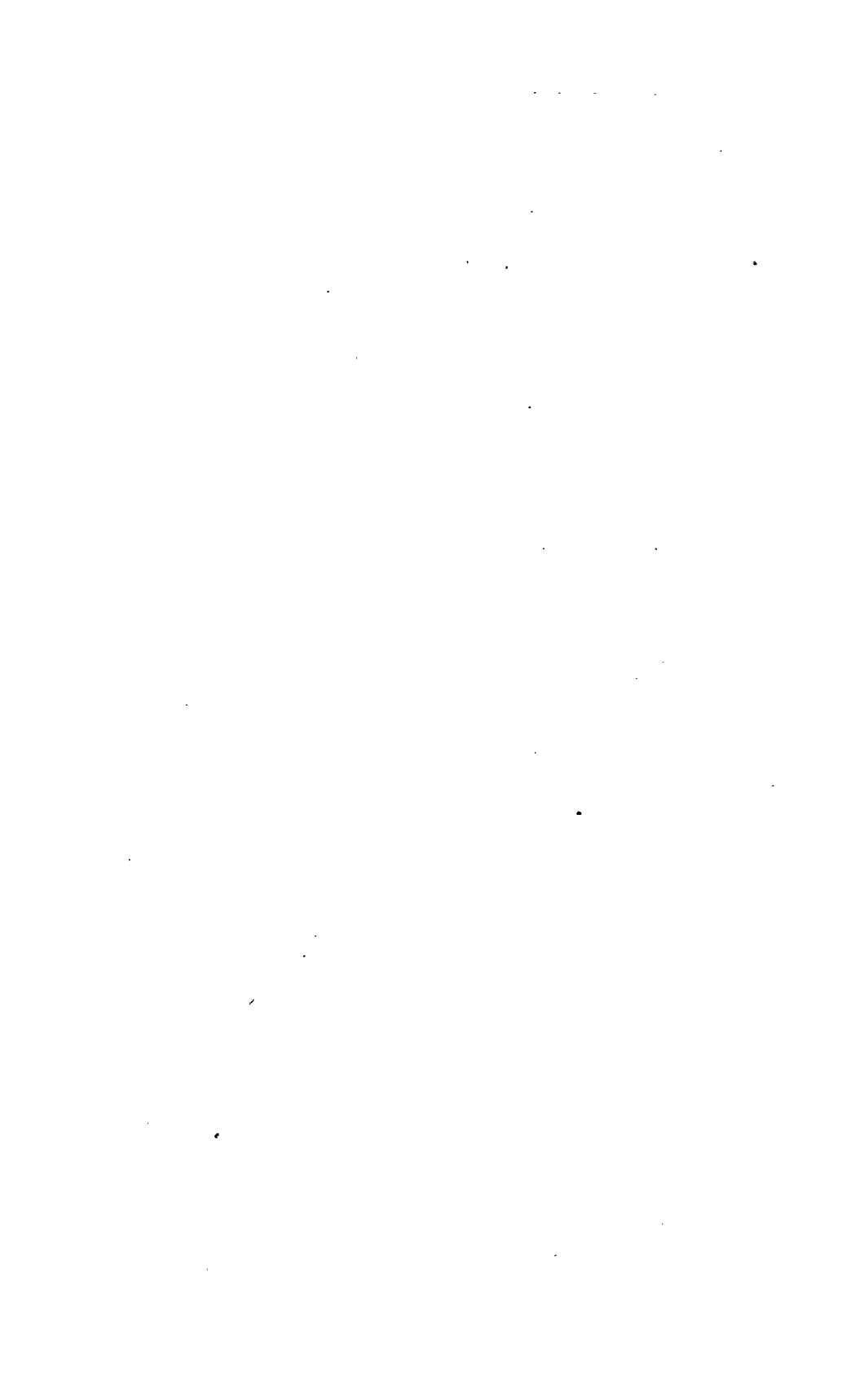
Practical cooking tests have shown that the canned product from freshly dug sweet potatoes is well adapted to a variety of table uses and, because of its firmness, is superior to that canned from cured stocks for the preparation of such dishes as sautéd and candied sweet potatoes.

Although many of the varieties studied gave an attractive product in the can, a few of them were found to have particular merit. Nancy Hall, Gold Skin, Vineless Pumpkin "Yam," and Porto Rico were among the best, but a very good canned product was obtained from Big Stem Jersey, Triumph, Red Bermuda, and others. It is important to note that those possessing the highest quality in the product of the freshly dug potatoes were also judged, in the earlier studies, as having particular merit when canned after the usual curing and storage treatment.

As a result of these combined studies it is apparent that by judicious handling of the raw stock the sweet-potato canner is able to put on the market products suited to every demand which the sweet potato supplies as a table commodity, and which for certain culinary uses is equal if not superior to the fresh vegetable.

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A LEAF SPOT OF CRUCIFEROUS PLANTS CAUSED BY *ALTERNARIA HERCULEA*¹

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INTRODUCTION

While studying the ring spot of cruciferous plants caused by *Mycosphaerella brassicicola* (Fr.) Lindau, another type of leaf spot was found to be common, especially in a vegetable section on the western side of San Francisco Bay in California, just south of the city of San Francisco. The disease was especially prevalent on cabbage (Columbo variety) and cauliflower (50 varieties) growing in the eastern part of this section in the autumn and winter of 1923-24. Since two other similar leaf-spot diseases also occurred in this locality, some observations and studies were made for the purpose of distinguishing between them. The object of this paper is to record the results of these studies.

SYMPTOMS OF THE DISEASES

Ring spot is very common on crucifers in the vegetable-growing section above referred to, known as the Colma section. Although this disease is described elsewhere (7),² a brief description is given here for convenience.

Infection first becomes evident on the leaves, regardless of age, as very small dark-colored spots one-half millimeter or less in diameter. These spots enlarge, and in 3 to 4 weeks there are lesions 1 to 2 centimeters in diameter. Both the upper and lower surfaces of mature lesions are thickly studded with pycnidia or perithecia or both, they being more abundant on the upper surface. The pycnidia and perithecia are often arranged in concentric circles; hence the name ring spot. Plate 1, A, shows the characteristics of the lesions. These lesions vary in color, but are usually sepia to clay-colored. However the pycnidia and perithecia are often so abundant that the spots appear almost black. There is usually a rather sharp line of demarcation between the diseased and healthy tissues, although the tissues immediately surrounding the lesions may be yellow ochre, gradually shading off to the normal green color. Nearly all parts of the host are susceptible.

Another leaf spot found in this section on various cruciferous hosts is caused by the fungus commonly known in this country as *Alternaria brassicae* (Berk.) Sacc.³ As stated in an earlier paper (6), this disease is not very prevalent in the San Francisco Bay section. However, it occurs at times and may be found on the same leaves with ring spot and the disease described below. This disease is known as

¹ Received for publication Feb. 8, 1926; issued October, 1926.

² Reference is made by number (italic) to "Literature cited," p. 650.

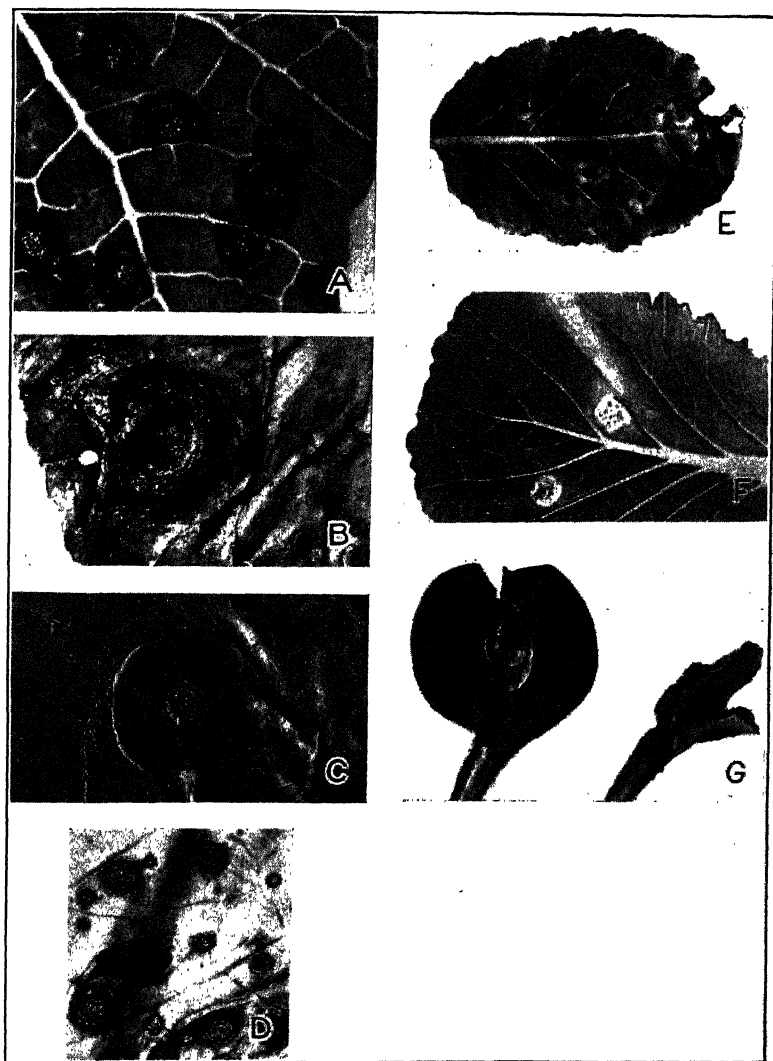
³ The name of this fungus has recently been changed to *Alternaria circinans* (B. & C.) Bolle by Bolle (8). It is not the purpose of this paper to go into a detailed discussion of nomenclature, hence the question as to whether or not Bolle is justified in making this change will be left open.

black leaf spot (4), *Alternaria* spot, or black mold. In its early stages it is indistinguishable from the dark spot caused by the ring-spot fungus. The spots spread rather rapidly, forming circular lesions varying in size from 0.5 to 1 cm. in diameter. The color varies from grayish to brown. Under conditions of high humidity the surfaces of the lesions become covered with sporophores and spores of the causal organism, which give them an olivaceous appearance. As in the case of ring spot, these fruiting bodies are arranged in concentric rings (pl. 1, B).

The third type of leaf spot found in this locality resembles the other two in size, shape, distribution over the leaf surface, and in presence of concentric rings. These latter are clearly shown in Plate 1, C and D. For convenience in this discussion, this disease will be designated as gray leaf spot, to distinguish it from black leaf spot and ring spot. The disease differs from ring spot in that the lesions are usually lighter in color and bear no pycnidia or perithecia. It is similar to the black leaf spot, in that under moist conditions the spots are covered with an olivaceous growth of the causal fungus (pl. 1, C). However, this growth is usually not as dense as is the case on the black leaf-spot lesions, and under field conditions in this locality it usually is not seen at all. Likewise, the lesions of the gray leaf spot are, on the average, smaller and lighter in color than are those of the black leaf spot (pl. 1, D). Gray leaf-spot lesions on other hosts in California have much the same appearance as on cauliflower and cabbage, except for a slight variation in color. On Chinese cabbage, radish, and turnip, the spots are light gray, while on cabbage and cauliflower they vary from gray to dark brown. As seen in the field they are usually some shade of gray.

DISTRIBUTION AND ECONOMIC IMPORTANCE

This disease is not a new one to science, neither does it have a limited distribution. It is known to occur in central and southern California, and in Florida, New York, Alabama, New Jersey, Indiana, Ohio, and Connecticut, and it probably is present elsewhere. In spite of this prevalence it apparently has been given little attention by pathologists, no doubt because it ordinarily causes little damage. It was, however, very prevalent on cauliflower and cabbage in certain parts of the Colma section in the autumn of 1923 and the winter of 1924. The ring spot was abundant near the ocean, but became decreasingly prevalent back from the coast; and the reverse was true of the gray leaf spot. The lower leaves of cauliflower were quite badly spotted with the latter disease in some fields on the eastern side of the Colma section. No doubt some of the lower leaves were much debilitated by the disease and the heads correspondingly decreased in size. Nevertheless, it is very difficult to determine losses produced by diseases of this nature. For the most part only about six of the lower leaves of the large plants were attacked, and the plants produced fair-sized heads. In no case was the disease severe enough to justify control measures.



- A.—Young cauliflower leaf affected with ring spot caused by *Mycosphaerella brassicicola*. The small black spots are lesions in early stages of development. 1.2X natural size.
- B.—Portion of a cauliflower leaf showing a large lesion of black leaf spot caused by *Alternaria brassicae*. 1.2X natural size.
- C.—Portion of cauliflower leaf showing a large lesion of gray leaf spot caused by *Alternaria herculea*. 1.2X natural size.
- D.—Section of cauliflower leaf affected with gray leaf spot caused by *Alternaria herculea*. [This type of lesion is more common than that shown in C. The concentric rings in these lesions are more pronounced than in the case of ring spot (pl. 1, A). The spots are usually smaller and lighter in color than are the black leaf-spot lesions (pl. 1, B).] About half natural size.
- E.—Young cabbage leaf showing gray leaf-spot lesions as they appeared thirteen days after inoculation. About half natural size.
- F.—Leaf of Chinese cabbage having two gray leaf-spot lesions produced by artificial inoculation. About half natural size.
- G.—Cotyledons of cauliflower seedlings artificially infected by *Alternaria herculea*. 1.8X natural size.



A head of cauliflower photographed one week after it was inoculated with *Alternaria herculea* and placed at 20° to 25° C. in a moist chamber. The black spots show the extent of infected areas. Two-thirds natural size

THE CAUSAL ORGANISM

The gray leaf spot is caused by the fungus first described by Ellis and Martin (3) on horse-radish (*Armoracia rusticana* Gaertn.) as *Alternaria herculea* (Ell. & Mart.) Elliott. Later Stewart (5) reported it as being common on flat turnip and found occasionally on cabbage on Long Island, N. Y. The fungus was first classed as a *Macrosporium*, but was later transferred to the genus *Alternaria* by Elliott (2), where, according to the present conception of the genus, it undoubtedly belongs.¹

Frequently it is necessary to make a microscopic examination of the spores before the black and gray leaf spots can be differentiated. Figure 1 shows how the spores differ in size and shape. The apical

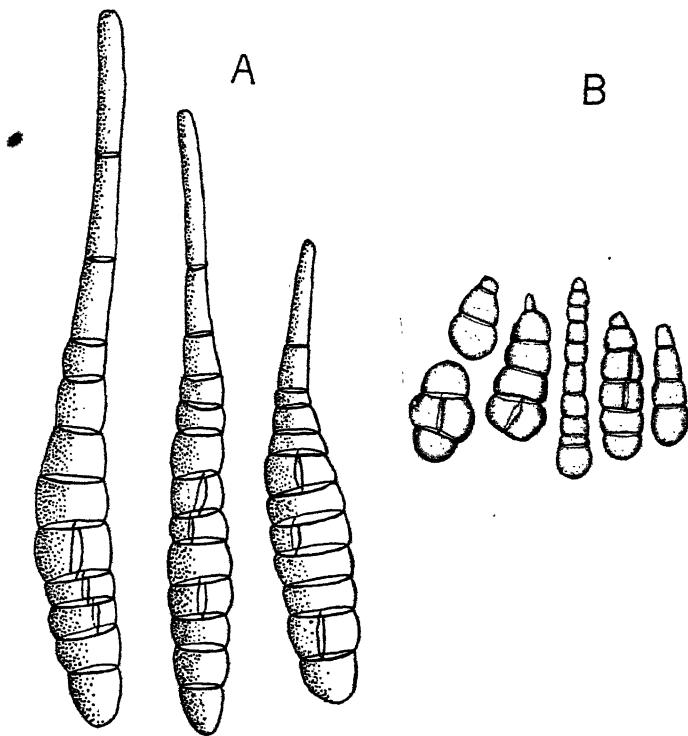


FIG. 1.—A. Three spores of *Alternaria herculea*. $\times 500$. B. Group of spores of *Alternaria brassicae*. $\times 500$

cell of an *Alternaria herculea* spore is characteristically long as compared with that of an *A. brassicae* spore. The spores of the former are also lighter in color, proportionately longer and narrower, and usually have more longitudinal septa than do those of the latter. *A. herculea* spores measure 16 to 28×125 to 225μ , while the largest spores of *A. brassicae* (5 to 8 septate) are 9 to 16.8μ wide by 35 to 75μ long (6).

¹ Bolle (1) has decided that the correct name of this fungus is *Alternaria brassicae* (Berk.) Bolle. However, the present writer is not entirely convinced that this change is justified, so for the purpose of this paper he prefers to use the name *A. herculea*.

Stewart (5) states that this fungus is undoubtedly parasitic on turnips, but cites no experiments to prove it. Since no proof of its pathogenicity could be found, a few tests were made on different hosts. Isolations were made from a number of typical lesions on cauliflower and *Alternaria herculea* was obtained in nearly every case. Inoculation tests on cauliflower, cabbage, and Chinese cabbages showed this fungus to be parasitic on them. The fungus was isolated from Chinese cabbage and from rutabaga-infected cabbage. Not only were the old leaves of cabbage infected, regardless of size, but the cotyledons were also. Cauliflower cotyledons only a few days old have been collected a number of times from seed beds at Colma and the fungus isolated. *Alternaria herculea* isolated from rutabaga leaves was used to inoculate cauliflower seedlings growing in the greenhouse, in the following manner: Hyphae in small amounts were placed on the cotyledons without consciously injuring them, and the plants were covered with battery jars. Three days later there was evidence of infection in the form of small, dark spots just visible to the unaided eye. In 12 days some of the cotyledons were almost entirely killed, while others showed quite distinct grayish-brown lesions having the concentric ring character (pl. 1, G). These lesions were very similar to those found on the cotyledons under natural conditions. In like manner, hyphae, from a culture of *A. herculea* from rutabaga were placed on the moistened leaves of Chinese cabbage seedlings (Wong Bok) growing in the greenhouse. These plants were kept moist under a bell jar for three days. At the end of that time water-soaked spots varying in size with the amount of the inoculum were present on the leaves. Plate 1, F, is a photograph of two of these lesions as they appeared at the end of 12 days. These spots were circular in outline, grayish, and varied from 1 mm. to 1 cm. in diameter. There was usually a sharp line of demarcation between the diseased and healthy tissues. The fungus fruited sparingly on the lesions, except when they were placed under conditions of high humidity. The fungus isolated from rutabaga reinfected the leaves of seedlings of this host (improved purple top variety) as well as cauliflower seedlings held in the greenhouse and inoculated as in the instances cited above. The fungus was reisolated from the lesions formed on rutabaga and used to inoculate Chinese cabbage with positive results. There is, therefore, considerable evidence that the fungus attacking all of these hosts is the same.

In one experiment conducted in the Pathological Garden at Berkeley, Calif., and designed to prove the pathogenicity of the ring-spot fungus, cauliflower leaves heavily infected with ring spot and also having some gray leaf-spot lesions, were suspended over each of 12 half-grown cauliflower plants. There was a heavy fog at the time, and this, together with some rain at intervals, kept the plants wet most of the time for three days and nights. Likewise, similarly affected leaves were suspended over some cabbage seedlings growing near. Six days later numerous small dark lesions were present on many of the cauliflower as well as the cabbage leaves. These proved to be due to *Alternaria herculea*, the lesions caused by the ring-spot fungus not being distinguishable as such until more than a week later. Under the conditions of the test, the *A. herculea* lesions developed much more rapidly than did those of the ring-spot fungus. Some of the

A. herculea lesions on the cabbage seedlings are shown in Plate 1, E, as they appeared 13 days after inoculation.

Since *Alternaria herculea* is so commonly present in some of the cauliflower fields in the Colma district, and as this cauliflower is held for several days in refrigerator cars and on the market before it reaches the consumer, the question arises as to what effect this fungus may have on the curd. To determine this point the following experiment was conducted. A medium-sized head of cauliflower was washed under the tap, placed in a battery jar lined with moist filter paper, and inoculated by placing on it in several places small amounts of hyphae from an 8-day old culture of *A. herculea*. The battery jar was then covered with a glass lid and held at room temperature (20° to 25° C.). A very slight amount of decay was observed on the third day after the inoculations were made. The decay developed slowly, as may be inferred from the condition of the head at the end of a week, as shown in Plate 2. It appeared from this experiment that *A. herculea* can cause a decay of the curd; but, under the conditions of the experiment, the rot progressed very slowly. The character of this rot and the appearance of the lesions are very similar to those caused by *A. brassicae*, as described in a former paper (6). The decay, however, does not develop as rapidly as does that caused by the latter fungus.

Another experiment was then conducted to determine whether any decay would occur at temperatures more nearly approaching those existing in a refrigerator car. Four heads were inoculated as in the above experiment, and held at temperatures of 0°, 2°, 4.5°, and 9° C., respectively. Observations made a month later showed that at 0° the fungus had caused a decay extending 2 to 3 mm. in depth, and covering an area slightly larger than the inoculum. The decayed tissues were quite soft and moist and were lighter in color than the surrounding healthy tissues. At 2° the fungus had decayed about 1 c. c. of the tissues upon which the inoculum had been placed. The infected tissues were brownish, soft and moist, and a microscopic study showed them to be filled with mycelium penetrating in every direction. The cellular structure was almost entirely disorganized. A few isolated cells were seen, but for the most part the tissues had been replaced by the fungus. The decayed tissues had an odor common to decaying cauliflower, but not the foul odor characteristic of bacterial rots of this host. At 4.5° and 9° C. the amount of decay was only slightly greater than that at 2°. *A. herculea* was recovered in pure culture from the decayed tissues at all four temperatures. This experiment, although on a very limited scale, indicates that this fungus can decay the curd of cauliflower, even at temperatures similar to those to which the cauliflower is subjected in the refrigerator car. However, the fungus develops slowly and ordinarily will not cause much damage to cauliflower during the time it is in transit.

SUMMARY

A leaf spot of cruciferous plants caused by *Alternaria herculea* (Ell. & Mart.) Elliott, which was quite prevalent in the vegetable section just south of San Francisco, Calif., in the season of 1923-24 is described and illustrated. This disease is quite similar to the leaf spot

caused by *Alternaria brassicae*, and is no doubt frequently confused with it.

The parasitism of the fungus on cabbage, cauliflower, Chinese cabbage, and rutabaga was proved.

Alternaria herculea can cause a slow decay of the curd of cauliflower at temperatures of 0°, 2°, 4.5°, 9°, and 20° to 25° C.

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HERITABILITY OF DIFFERENT RATES OF SHEDDING IN COTTON¹

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INTRODUCTION

The shedding of flower buds and young bolls of cotton has been the subject of several investigations in which shedding has been considered from the physiological point of view and with especial reference to the influence of environmental factors. A survey of the literature of this phase of the subject would be out of place here, but publications by Balls (1, p. 67-75²), Thornton (23), Lloyd (15, 16), Harland (5), Ewing (4), Cook (3), McKillop (17), Mason (18, 19), Jivanna Rao (12), Prescott (22), Zaitzev (24), and Hilson (11) may be cited in this connection.

Shedding has been little studied from the point of view of heredity, but there are published data which make it reasonably certain that genetic factors are involved. Harland (5, p. 78), summarizing observations made by him on the island of St. Vincent, W. I., states:

In hybrids between types differing in the bud-shedding habit, it has been established that when sea island is crossed with a West Indian native in which the bud-shedding habit is developed, the F_1 exhibits this bud-shedding. * * * Crosses between upland and West Indian native give rise to an F_1 which does not shed its buds.

Ewing (4, p. 64, 66) found differences in the rate of shedding of different varieties of upland cotton. Cook (3) remarks:

A genetic factor is plainly indicated in plants that abort all of their buds, while their neighbors mature good crops. Egyptian cotton may retain nearly all of its buds and young bolls while upland varieties in adjacent rows are shedding nearly all of their buds.

Patel (20), comparing six strains of a variety cultivated in India and referred by him to *Gossypium herbaceum* L., found that the three strains which gave the highest percentages of bud shedding in 1920-21 also gave the highest percentages in 1921-22. The one strain which was highest of all in bud shedding in both seasons also ranked highest in percentage boll shedding in three successive seasons.

The comparative boll shedding of the Pima variety, representing the Egyptian type of cotton, and of several varieties of the upland type, was studied by King, Loomis, and Varmette (14, p. 943) at the United States Field Station, Sacaton, Ariz. In this case the comparison is between different species, Egyptian cotton belonging to the group containing sea island cotton (*G. barbadense* L.) and the upland varieties belonging to *G. hirsutum* L. King and his coworkers state in regard to their findings:

Pima cotton in 1922 retained 82.5 per cent of its bolls and in 1923 70.7 per cent, under conditions where upland varieties usually retained less than 30 per cent of their bolls.

¹ Received for publication Mar 19, 1926; issued October, 1926.

² Reference is made by number (italic) to "Literature cited," p. 660.

The existence of a difference of this magnitude between the two types is not surprising in view of the differences in tissue-fluid properties discovered by Harris and his coworkers (6, 7, 8, 9) and the numerous and striking morphological differences between Egyptian and upland cottons, some of which have been summarized by Kearney (13, p. 4-6).

A study of hybrids between Pima Egyptian and Acala upland cotton, grown at Sacaton, Ariz., in 1925, has given additional evidence that there are genetic variations in shedding. The comparative shedding rates of the parental types will first be considered.

COMPARATIVE SHEDDING OF PIMA EGYPTIAN AND ACALA UPLAND COTTONS

Shedding records were kept on plants of Pima and of Acala cotton grown under as nearly as practicable identical conditions at the United States Field Station, Sacaton, Ariz., in 1923, 1924, and 1925, and at the United States Cotton Field Station at Shafter, Calif., in 1923.³

The records from which were computed the shedding percentages took account only of primary or extra-axillary buds and flowers borne on the fruiting branches of the main stalk. Secondary or axillary buds and flowers, such as appear at many nodes of the fruiting branches, were ignored, as well as all buds and flowers borne on the vegetative branches or limbs. The justification for this procedure is that individual plants differ greatly in the production of vegetative branches and of axillary flower buds on the fruiting branches, and the shedding of buds and young bolls borne in these positions seems to be more readily affected by slight variations of the environment than is the case with those borne in the extra-axillary position on the fruiting branches of the main stalk.⁴

The seasonal shedding percentages, as given in Table 1, were computed from the total number of flower buds produced and shed and the total number of flowers produced and shed (as young bolls) by all plants in each population, taken as one array. The standard deviations and probable errors of the mean percentages were computed from the squared departures of the individual plant percentages from the mean percentage of the whole population, each squared departure having been weighted for number of buds or of flowers on the plant in question.

It is evident from the data in Table 1 that the percentage of bud shedding (abscission before anthesis) of the two types did not differ significantly either in 1924 or in 1925. But in percentage of boll shedding (abscission after anthesis) the two types differed greatly and very significantly in all four comparisons. The Acala percentage was from 4.2 to 7.4 times the Pima percentage and the difference was from 14 to 35 times its probable error.

³ The records were kept at Sacaton in 1923 by Max Willett and Dow D. Porter, of the Office of Alkali and Drought Resistant Plants; at Shafter in 1923 by Robert H. Peebles, of the same office; and at Sacaton in 1924 and 1925 by Harold F. Loomis, of the Office of Acclimatization and Adaptation of Crop Plants. The writers are indebted to O. F. Cook for the use of Mr. Loomis's data.

⁴ C. J. King has called the writers' attention to the fact that in the Pima variety, at least, the effective shedding, as shown by the number of vacant nodes on the fruiting branches at the end of the season, is much less than is indicated by the shedding percentages based on extra-axillary buds only, since bolls develop from secondary or axillary buds at many of the nodes from which the primary or extra-axillary bud had been recorded as shed. This observation is important from the point of view of ultimate yield but does not affect the validity of the comparison of the two types of cotton in this paper.

TABLE 1.—Seasonal shedding percentages of Egyptian cotton (*Pima variety*) and upland cotton (*Acala variety*) in 1923, 1924, and 1925 ^a

Variety	Year	Station	Bud shedding			Boll shedding		
			Number of plants	Number of buds	Percentage shed	Number of plants	Number of flowers	Percentage shed
Pima.....	1923	Sacaton, Ariz.....	20	1,317	39.4±0.89	40	1,565	15.5±1.48
Acala.....	1923	do.....				40	2,340	69.4±1.37
Difference.....								53.9±2.02
Pima.....	1923	Shafter, Calif.....	20	955	52.1±1.03	30	590	9.3±1.35
Acala.....	1923	do.....				40	1,240	68.9±1.01
Difference.....								59.6±1.69
Pima.....	1924	Sacaton, Ariz.....	20	1,445	37.4±0.99	20	904	19.1±1.71
Acala.....	1924	do.....	20	1,884	36.2±1.36	20	1,202	80.7±1.11
Difference.....					1.2±1.68			61.6±2.04
Pima.....	1925	Sacaton, Ariz.....	20	1,791	43.9±1.19	20	1,004	11.2±3.62
Acala.....	1925	do.....	20	1,796	47.4±1.27	20	945	66.7±1.83
Difference.....					3.5±1.74			55.5±4.06

^a Bud shedding was not recorded on the Acala plants in 1923.

COMPARATIVE SHEDDING IN F₁ AND F₂ OF THE HYBRID PIMA × ACALA

The material representing this interspecific hybrid consisted of 10 first-generation and 91 second-generation plants, all grown in the same plot at the United States Field Station, Sacaton, Ariz., in 1925. The F₁ plants were near the south end of row 1. Of the F₂ plants 68 were scattered through the entire length of row 2, and 23 were in the south one-third of row 3. As was the case in recording the shedding of the parental types, only the primary or extra-axillary flower buds borne on the fruiting branches of the main stalk were considered.

All flowers opening during the period from June 17 to September 8 (84 days) were tagged daily and a daily record of bolls shed was kept for each plant. From these data the seasonal boll-shedding percentage (bolls shed as a percentage of total flowers) was computed for each plant. After the daily recording was terminated, counts were made on each plant of the total number of nodes on the fruiting branches of the main stalk and of the number of nodes at which the primary or extra-axillary bud or boll was missing. Subtraction from the latter number of the number of nodes at which shedding had occurred after anthesis, as shown by the daily records of boll shedding, gave the number of buds shed before anthesis, and this number, relative to the total number of nodes on the fruiting branches, gave the seasonal percentage bud shedding for each plant.

The frequency distributions of the shedding percentages (in classes of 5 per cent each) of the F₁ and F₂ populations are given in Table 2 and are shown graphically in Figure 1. The mean shedding percentages and their standard deviations, for each population, are stated in Table 3. The means were computed from the percentages of the individual plants, not from the distributions by classes as

given in Table 2. The standard deviations and probable errors were computed from the departures of the individual plant percentages from the mean percentage of the population.⁵

TABLE 2.—Frequency distributions of percentage of bud shedding and percentage of boll shedding on 10 F_1 plants and 91 F_2 plants of Pima×Acala

Percentage	Number of plants				Percentage	Number of plants			
	Bud shedding		Boll shedding			Bud shedding		Boll shedding	
	F ₁	F ₂	F ₁	F ₂		F ₁	F ₂	F ₁	F ₂
0-5					51-55		5		11
6-10	4	2			56-60		1		8
11-15	5	8		2	61-65				6
16-20		7	2	5	66-70		1		5
21-25	1	8	4	3	71-75				2
26-30		14	3	6	76-80				4
31-35		12	1	10	81-85		1		2
36-40		11		7	86-90				2
41-45		10		10	91-95				1
46-50		11		9	96-100				1

TABLE 3.—Mean bud and boll shedding percentages and their standard deviations in F_1 and F_2 populations of Pima×Acala

Number of plants	Generation	Bud-shedding percentage		Boll-shedding percentage	
		Mean	Standard deviation	Mean	Standard deviation
10	F_1	12.9±1.42	6.66±1.00		5.64±0.85
91	F_2	27.2±1.28	18.17±.91	23.7±1.20 47.2±1.29	18.30±.91
Difference.....		14.3±1.91	11.51±1.35	23.5±1.76	12.66±1.24
D/E *		7.5	8.5	13.3	10.2

* Difference divided by its probable error.

A comparison of the mean shedding percentages of the hybrids (Table 3) with those of the parent varieties in 1925 (bottom section of Table 1) is not very satisfactory, owing to the fact that the parental and the hybrid populations were grown in different fields. It is true that the plants of all four populations made an excellent growth, but the fact that the bud shedding of both Pima and Acala was approximately 45 per cent, whereas in F_1 and F_2 of the hybrid it was only 13 and 27 per cent, respectively, indicates a radical difference in the conditions under which the parents and hybrids were grown. The relative boll shedding of the four populations is more in line with the expectation, the mean percentages having been: Pima, 11; F_1 , 24; F_2 , 47; Acala, 67. In this case the means of both hybrid generations were between those of the parents.

⁵ The standard deviations of the mean percentages have been increased by using Pearson's correction for the standard deviation of small numbers (21, p. 525). A further increase of the standard deviation has been made on the basis suggested by Collins and Kempton (2), who present the matter as follows: "Since the fluctuations in ratios due to errors of sampling become smaller as the ratio departs from 0.5 and the change is measured by the change in \sqrt{pq} , the observed standard deviations have been multiplied by

$\frac{5}{\sqrt{pq}}$. This changes the standard deviation to that expected had the ratio been 0.5 or 50 per cent." The probable errors were computed from the standard deviations as thus corrected.

Comparing the mean shedding percentages of the two hybrid generations as given in Table 3 it is seen that the rate of shedding, both of buds and bolls, was approximately twice as great in F_2 as in F_1 . The difference F_2-F_1 is very significant in both cases, having been, respectively, 7.5 and 13.3 times its probable error. Comparison of the standard deviations of the two generations shows F_2 to have been nearly three times as variable as F_1 in bud shedding and more than three times as variable in boll shedding. Here again the difference F_2-F_1 is very significant, having been, respectively, 8.5 and 10.2 times its probable error.

The F_2 frequency distributions for percentage bud shedding and percentage boll shedding (Table 2 and Figure 1) give no indication of segregation in definite ratios, the presumption being that several genetic factors are involved. Shedding therefore behaves like most of the morphological characters in Egyptian-upland cotton hybrids (13, pp. 15-26, figs. 3-41), and the inheritance is of the type usually observed in size characters.

A strong presumption that there was Mendelian segregation in respect to shedding is created by the fact that the second generation was so much more variable than the first. The individual bud-shedding percentages ranged from 6 to 82 in F_2 but only from 8 to 22 in F_1 . The boll-shedding percentages ranged from 13 to 100 in F_2 but only from 16 to 31 in F_1 . In respect to both percentages the standard deviation was approximately three times as great in F_2 as in F_1 .

Further evidence of segregation might have been had by comparing the shedding performance of the two hybrid generations with that of the inbred Pima and Acala families to which the parents of the hybrid belonged. Small progenies of both families were grown in 1925, but through an oversight shedding data were not obtained from them. Comparison with the Pima and Acala populations which afforded the data given in the bottom section of Table 1 would be of no use in this connection, because these populations were of the general commercial stocks of these varieties and, moreover, were grown in a different field from that in which the hybrid populations were located.

It has been mentioned that whereas the F_1 plants were located near the south end of row 1, the F_2 plants were distributed throughout the

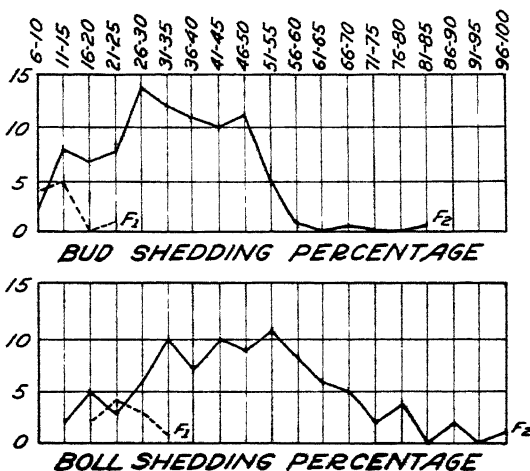


FIG. 1.—Frequency distributions of percentage of bud shedding and percentage of boll shedding of 10 F_1 plants and 91 F_2 plants of the cotton hybrid Pima x Acala. The figures on the ordinate indicate the numbers of plants in the respective class, and the figures on the abscissa indicate the shedding percentages in classes of 5 per cent each. The dotted lines represent the F_1 distributions and the solid lines the F_2 distributions.

length of row 2 and the south one-third of row 3. Because of the recognized importance of soil heterogeneity at the Sacaton station (10, p. 609-612), this wider distribution of the plants may well have been a factor in the much greater variation in the F_2 . To test this point, the statistical constants were separately computed for 12 F_2 plants which were located at the south end of row 2 and occupied a section exactly coterminous with that occupied by the 10 F_1 plants in the adjacent row 1. The constants for this portion of the F_2 population, in comparison with F_1 , are given in Table 4.

TABLE 4.—Comparison of the statistical constants of the F_1 population and of a portion of the F_2 population of Pima \times Acala, the plants of the two generations occupying adjacent and coterminous sections of rows

Shedding percentages	Generation	Mean	Standard deviation
Percentage of bud shedding.....	{ F_1	12.9 \pm 1.42	6.66 \pm 1.00
	{ F_2	15.7 \pm 2.29	11.75 \pm 1.61
	Difference.	2.8 \pm 2.69	5.09 \pm 1.89
	D/E ^a	1.0	2.7
Percentage of boll shedding.....	{ F_1	23.7 \pm 1.20	5.64 \pm .85
	{ F_2	48.8 \pm 2.08	10.71 \pm 1.47
	Difference.	25.1 \pm 2.40	5.07 \pm 1.70
	D/E.....	10.4	3.0

^a Difference divided by its probable error.

A comparison of the means of these 12 F_2 plants with those of the whole F_2 population of 91 plants (Table 3) shows that the mean percentage of bud shedding is little more than half as great as in the whole population and the difference is significant (D/E 4.4). On the other hand, the mean percentage boll shedding of the sample does not differ significantly from that of the whole F_2 population.

The standard deviation of the mean bud-shedding percentage is only 65 per cent and that of the mean boll-shedding percentage is only 59 per cent as great in the sample as in the whole F_2 population. This fact indicates that soil heterogeneity may have been responsible for part of the variability in F_2 shown by the frequency distributions in Table 2 and the standard deviations in Table 3.

Returning to the comparison of adjacent plants of the two generations, as set forth in Table 4, in which comparison soil heterogeneity presumably is eliminated, the mean bud-shedding percentage is not significantly higher in F_2 than in F_1 , but the mean boll-shedding percentage is very significantly higher in F_2 , the difference being 10.4 times its probable error. The standard deviations of the mean percentages show F_2 to have been about twice as variable as F_1 , both in bud shedding and in boll shedding, although the differences are barely significant. But in view of the small numbers of the populations and the resulting large probable errors of the standard deviations, significant differences were hardly to have been expected.

While the position of the F_1 plants in an outside row may have given them a slight advantage, the adjacent F_2 plants made an excellent growth and it is unlikely that this difference in environment could have been a material factor in the heavier rate of boll shedding and in the greater variability in both bud shedding and boll shedding of

the F_2 population. On the whole, the evidence from this closer comparison of F_1 and F_2 does not seriously weaken the conclusion that genetic factors are involved in the shedding performance of this hybrid.

GENETIC VARIATION SHOWN BY COMPARING ADJACENT F_2 PLANTS

Further evidence as to whether genetic factors are concerned in shedding was sought by comparing the shedding percentages of pairs of F_2 plants, the two plants in each comparison having been adjacent in the row. This procedure virtually eliminates the influence of soil heterogeneity. The Pima \times Acala F_2 population afforded 56 such comparisons, involving 79 of the 91 plants.⁶ Significant differences (difference three or more times its probable error) were shown by 30 of the 56 pairs in percentage of bud shedding and by 28 pairs in percentage of boll shedding.

A means of expressing concisely what may be termed the non-fortuitous variation in a population is afforded by comparing the standard deviation computed from the mean squared differences between pairs with the standard deviation expected if only chance variation had occurred. Since in the observed standard deviation as thus computed the effect of soil heterogeneity is virtually eliminated, the amount by which it exceeds the expected standard deviation may be taken to represent the amount of variation due to genetic factors.

The observed and expected standard deviations were computed for the percentages of bud shedding and boll shedding in the Pima \times Acala F_2 population of 1925. Also, for comparison, these standard deviations were computed for a population of 100 plants of Pima cotton, consecutive in the row, on which shedding was recorded at Sacaton in 1922. Eleven of the 99 pairs of Pima plants differed significantly in respect to bud shedding and 10 pairs in respect to boll shedding.

The standard deviations based upon these comparisons of paired plants are given in Table 5.

In Pima \times Acala F_2 the observed standard deviation is about 3 times the expected standard deviation, for both the bud-shedding and the boll-shedding percentage, and the difference in both cases is highly significant, being about 10 times its probable error. It is clear that the individual differences in shedding in this population are largely of a genetic nature.

In the relatively homogenous Pima population, on the other hand, the observed standard deviation only slightly exceeds the expected standard deviation for the bud-shedding and boll-shedding percentages, but the difference in each case appears to be significant (about 3.5 times its probable error).⁷

⁶ Numerous plants on which flowers had been bagged for self-fertilization were scattered through the row and these were not used; hence the reduced number of comparisons.

⁷ The correlation of adjacent plants in respect to percentage of bud shedding was positive in both populations. In Pima \times Acala F_2 the coefficient was not significant, having been $+0.167 \pm .087$ (1.9 times the probable error). It is not unlikely that the pronounced differences of a genetic nature in this case obscured the effect of soil heterogeneity. In the Pima population the coefficient was $+0.201 \pm .095$ (3.1 times the probable error). The low correlations in both populations indicate that soil heterogeneity was not very pronounced where they were grown.

TABLE 5.—*Observed and expected standard deviations of the mean shedding percentages in Pima × Acala F₂ of 1925 and in a Pima population of 1922*

Standard deviation	Pima × Acala F ₂ (79 plants, 56 paired comparisons)		Pima (100 plants, 99 paired comparisons)	
	Mean percentage of bud shedding	Mean percentage of boll shedding	Mean percentage of bud shedding	Mean percentage of boll shedding
Observed ^a	15.11±0.96	20.38±1.30	5.32±0.255	5.32±0.255
Expected ^b	4.93	7.10	4.40	4.46
Difference.....	10.18±.96	13.28±1.30	.92±.255	.86±.255
D/E.....	10.6	10.2	3.6	3.4

^a The formula is: $\sigma = \sqrt{\frac{\sum (d^2 \times \frac{1}{E^2})}{\sum \frac{1}{E^2}}} \div \sqrt{2}$ Where d = the difference between members of a pair and E = the probable error of the difference.

^b The formula is: $\sigma = \sqrt{\frac{pq}{H}}$, where p is the observed shedding percentage of the population (as one array) $q = 100 - p$ and H the harmonic mean number of buds (or flowers). H is computed by the formula $\frac{1}{\sum \frac{1}{n}} \div N$ where n is the number of squares (or flowers) on each plant and N the number of plants involved in the series of pairs

CORRELATION BETWEEN BUD SHEDDING AND BOLL SHEDDING

Correlating, for the 91 plants of Pima × Acala F₂, grown in 1925, the percentage bud shedding with the percentage boll shedding, a value for r of $+0.201 \pm .068$ was obtained. The coefficient is doubtfully significant, being slightly less than three times its probable error. The bud shedding and boll shedding percentages of 100 Pima plants at Sacaton, Ariz., in 1922 and of 44 Pima plants at Shafter, Calif., in 1922 gave coefficients of correlation of $-0.116 \pm .067$ and $-0.231 \pm .096$, respectively, neither coefficient being significant.

A negative correlation between bud shedding and boll shedding might be expected on physiological grounds, since if a certain amount of self-pruning is assumed to be necessary, heavy shedding of buds ought to favor the retention of most of the young bolls. On the other hand, a positive correlation might be expected on genetic grounds, since plants possessing factors conducive to abscission would probably show this tendency both before and after anthesis. Although none of the above coefficients of correlation appears to be significant with respect to its probable error, the fact that the two Pima populations of 1922 gave negative correlations indicates that here the physiological effect outweighed the genetic, while the positive correlation shown in F₂ of the Pima × Acala hybrid denotes a greater influence of genetic than of physiological factors in this population.

SUMMARY AND CONCLUSIONS

An interspecific cotton hybrid, Pima Egyptian × Acala upland, was studied for the purpose of ascertaining whether genetic factors are involved in the shedding of the flower buds and the young bolls (abscission before and after anthesis).

Comparison of populations representing the parental types showed practically no difference in the mean percentage of bud shedding but a consistently much higher rate of boll shedding in Acala than in Pima.

In both the first and second generations the hybrid gave a much lower mean percentage of bud shedding than either parental population, while the mean percentage of boll shedding of the hybrid in both generations was between the mean percentages of the parental types.

Comparing the two generations of the hybrid, the mean shedding percentage, both of buds and of bolls, was about twice as great in F_2 as in F_1 and the differences were very significant. Comparison of the standard deviations of the mean percentages showed F_2 to have been approximately three times as variable as F_1 and these differences also were highly significant.

Part of the greater variability in the second generation of the hybrid may have been due to the fact that the much larger F_2 population was exposed to a greater range of soil variation than the F_1 population. When this factor was eliminated by comparing the F_1 population with an approximately equal number of F_2 plants grown under the same soil conditions, the difference between the variability of the two generations was reduced but the standard deviations of F_2 were still about twice as great as those of F_1 .

The frequency distributions of the F_2 population for percentage of bud shedding and percentage of boll shedding, give no indication of segregation in definite ratios. The behavior, which resembles that of most size characters in plants and animals, may be interpreted on the theory that three or more genetic factors are involved. That Mendelian segregation nevertheless occurred, is suggested by the fact that the variation was much greater in F_2 than in F_1 .

Further evidence of genetic factors in shedding was obtained by comparing the shedding percentages of pairs of adjacent F_2 plants, the influence of soil heterogeneity being practically eliminated by this procedure. With 56 such pairs, significant differences were found in 30 cases in respect to bud shedding and in 28 cases in respect to boll shedding.

A comparison of the observed standard deviation computed from the mean squared differences between pairs with the standard deviation expected if only chance variation had occurred, showed that in Pima \times Acala F_2 the observed was about three times the expected standard deviation in both bud shedding and boll shedding and the differences in both cases were highly significant; whereas, in a population of 100 plants of the Pima variety the observed standard deviation was only 1.2 times the expected standard deviation both in bud shedding and in boll shedding. The great disparity between the size of the observed and the expected standard deviations of the hybrid is convincing evidence of genetic differences among the individual F_2 plants.

The correlation between the percentage of bud shedding and the percentage of boll shedding of the Pima \times Acala F_2 population was found to be positive, although scarcely significant, while in two Pima populations very small and probably not significant negative correlations were found. A positive correlation would be expected on genetic grounds, since genetic factors conducive to a heavy rate

of shedding presumably would operate both before and after anthesis. On the other hand, from a physiological point of view a negative correlation would be anticipated, since, assuming a certain amount of self-pruning to be necessary, a heavy rate of shedding before anthesis should be favorable to the retention of a greater proportion of the young bolls.

Differences in the rate of shedding of different types and varieties of cotton have been pointed out by previous investigators. Such differences indicate that shedding is, in part, a genetic phenomenon. The investigation described in this paper supplied the evidence that there are genetic factors for shedding which segregate and recombine in the usual manner.

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2000

INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEET POTATOES IN STORAGE¹

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INTRODUCTION

Black rot of sweet potatoes (*Ipomoea batatas*) is of considerable economic importance as a storage disease and may be responsible for some loss in transit and in the market when conditions are favorable for infection and development.

Black rot (*Ceratostomella fimbriatum* (E. & H.) Elliot) has its origin in the field, but the spores of the fungus may be carried into the storage house on the roots of the potato. This disease can be largely eliminated from the field through seed selection, disinfection, rotation of crops, and changing of the soil and manure (when manure is used for the purpose of heating) in the seed bed. Since, however, the disease is probably present in every region where sweet potatoes are grown, it continues to be a source of loss in the storage house and in the markets.

Where loss occurs in storage it may range from a fraction of 1 per cent to nearly 100 per cent. Poole² estimated a storage loss of 75 per cent in some instances where the field losses were confined to a few scattered hills. No figures to show the total loss throughout the country are at present available. In fact there seems to be no practical method known by which this loss can be accurately determined.

Although sweet potatoes may show little or no sign of black rot at digging time, the disease may develop and spread in the storage house.³ The rapidity with which this occurs depends upon the agencies by which it is spread and the conditions in the storage house and the railroad car. The means by which it is spread have not been studied, but the following are listed as probable: Contact, air movement, rodents, insects, and man. Temperature and humidity are among the most important factors affecting its development.

In the present paper a consideration is given to the method of infection, the influence of temperature on infection, the development of the disease, and the growth of the pathogene in culture.

MATERIALS

A pure-line strain of *Ceratostomella fimbriatum* was employed in these experiments. It was grown on corn meal and incubated at room temperature.

¹ Received for publication Apr. 27, 1926; issued October, 1926.

² POOLE, R. F. SWEET POTATO DISEASES IN NEW JERSEY. N. J. Agr. Expt. Sta. Circ. 141, 31 p., illus. 1922.

³ HARTER, L. L., WEIMER, J. L., and ADAMS, J. M. R. SWEET-POTATO STORAGE-ROTS. Jour. Agr. Research 15: 337-368, illus. 1918.

Little Stem Jersey was the variety of sweet potato used. The potatoes were cured at temperatures ranging from 25° to 30° C. and stored at temperatures between 10° and 15°.

EXPERIMENTAL DATA

The data will be presented under seven headings: (1) Growth of the pathogene, (2) method of infection, (3) time required for infection, (4) influence of temperature on the development of the disease, (5) influence of time on the development of the disease, (6) influence of temperature and time on the number of infection, and (7) temperature limits of infection.

GROWTH OF THE PATHOGENE

Erlenmeyer flasks (200 c. c. capacity) of sweet-potato agar were inoculated with a water spore suspension. By means of a platinum loop a small drop of the suspension was placed at the center of the culture medium in each flask. The flasks were then placed in infection chambers⁴ at different temperatures. The cultures were allowed to grow until those at the optimum temperatures had nearly reached the margin of the medium, so as to permit as much growth as possible at the border temperatures. This arrangement was desirable in order to compare the growth of the organism over a wide range of temperatures at a given time. It was not possible to bridge the entire temperature growth range with the size of flasks used. At 12° C. and below and at 34.5° and above little or no growth occurred in 16 days, the time required for the organism almost to cover the culture medium at the optimum temperatures. The cultures held at these temperatures were retained until a later date to allow for any further development.

The measurements were made by means of special calipers devised for that purpose.⁵ They consisted of two metal plates drawn out to a point at the ends and fastened at the centers, so that they could open and close like a pair of scissors. These were inserted in the flasks when closed and then spread out so that the two points were at opposite margins of a colony. The distance was then measured between the points at the opposite end of the calipers.

The results recorded in Table 1 represent the average of two experiments. No growth took place at 40° C. in 16 days and none at 36° in either 16 or 40 days. Some growth occurred at 34.5° in 16 days. This temperature is very near the maximum, as is indicated by the large decrease in growth which occurred as the temperature was raised from 33.5° to 34.5°. There was growth in 5 of the 12 cultures at 33.5° in 16 days. Two of the cultures showed considerable growth and three but little. These three, together with the seven which had shown no growth, were retained at the same temperature for 24 days longer. At the end of the 24 days two of the three were contaminated, but the third showed an area of only 2 square mm. Two of the seven were also contaminated, but the other five showed no growth.

⁴ LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. *Jour. Agr. Research* 24: 441-456, illus. 1923.

⁵ The calipers were devised by George F. Taylor, of the Office of Horticulture, U. S. Department of Agriculture.

TABLE 1.—Growth of *Ceratostomella fimbriatum* on sweet-potato agar at different temperatures

Number of flasks inoculated	Number of colonies measured	Length of growth period	Temperature	Average area of lesions	Remarks
		Days	° C.	Sq. mm.	
5	0	16	40.0	0	No growth in 3 flasks; 2 contaminated.
12	0	16	36.0	0	
5	1	16	34.5	5	
12	5	16	33.5	155	No growth in 7 flasks; growth in 5.
12	12	16	31.5	950	
17	16	16	28.5	1,782	
10	8	16	27.0	1,951	2 cultures contaminated.
16	15	16	24.5	1,929	1 culture contaminated.
16	14	16	23.0	1,884	2 cultures contaminated.
16	12	16	20.0	1,212	4 cultures contaminated.
16	12	16	18.5	766	Do.
10	9	16	14.0	61	1 to 2 mm. of growth in all cultures.
11	-----	16	12.0	-----	
11	-----	16	9.5	0	
11	-----	16	8.0	0	No growth in 9 cultures; 4 cultures contaminated.
11	-----	40	36.0	0	
10	1	40	33.5	2	
11	8	55	12.0	65	No growth in 5 cultures; 4 cultures contaminated.
11	0	55	9.5	0	3 cultures contaminated.
					No growth in 9 cultures; 1 contaminated; 1 doubtful colony.
11	0	55	8.0	0	4 cultures contaminated.
5	1	73	14.0	3,019	
5	2	73	11.5	647	
5	0	73	10.0	-----	2 cultures contaminated.
					Just a speck in 2; 1 culture contaminated; no growth in 2.

The lowest temperature at which there was any evidence of growth was 9.5° C. The growth at 10° was scarcely visible after 73 days. The greatest growth in 16 days occurred at 27° C., but it was only slightly greater than that at 24.5°. Some variation was noted in the results of the two experiments, the greatest growth in one occurring at 28° and in the other at 27°. The difference in the amount of growth which occurred between 23° and 28.5° was small. It may be said, therefore, that the optimum is represented by this range of temperature.

There was a marked increase in the growth of the fungus with the rise in temperature from 14° to 23° C. This augmented growth was due to two factors, namely, temperature and the area of the medium exposed to the action of the fungus. Since the growth of a colony takes place chiefly at the periphery, the area of growth is closely proportional to the circumference as long as the margin of the medium is not reached. Since the colony is larger at the higher temperature at any moment after growth begins, the area of growth is larger.⁶ This difference in area is progressively augmented with the lapse of time. Thus we have two variable factors, varying in the same direction and combining in a greatly increased rate of growth with a rise in temperature. This relation operates in the opposite direction as we pass from the optimum temperature to the maximum. The range of temperature, however, is narrower. The relation of growth of the fungus to rise in temperature is graphically represented in Figure 1.

METHOD OF INFECTION

The data relating to the method of infection were obtained from two experiments. In the first the potatoes were inoculated by

⁶ LAURITZEN, J. I., and HARTER, L. L. THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS. Jour. Agr. Research 30: 793-810, illus. 1925.

dipping in a spore suspension of the fungus. They were then placed in battery jars and distributed to various temperatures. After 17 days 123 lesions on potatoes that had been exposed to various temperatures were examined to determine whether or not infection had taken place through the uninjured skin. In all doubtful cases they were examined under the hand lens to make sure of the presence or absence of defects at the point of infection. Seven lesions showed a complete absence of any sign of defect or disturbance in the skin at the point of infection; 2 showed very slight abrasions; 70 showed

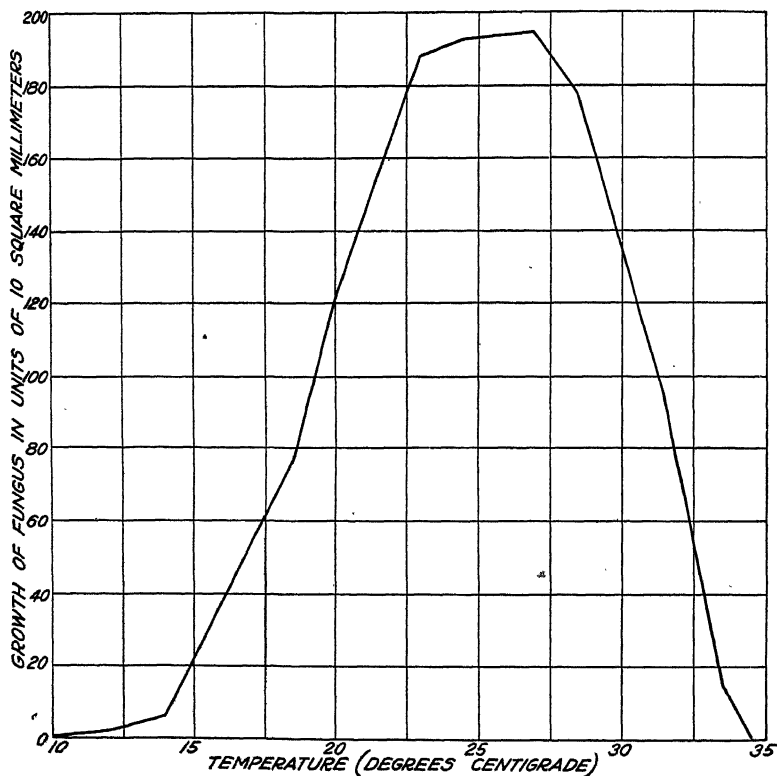


FIG. 1.—Curve showing the increments of growth of *Ceratostomella fimbriatum* with the rise in temperature after 16 days

abrasions or apparent disturbances at the center. In 41 cases infection occurred where rootlets emerged from the potatoes and in 3 cases it occurred through wounds.

In the second experiment the potatoes were inoculated as in the first, but were stored at various temperatures in wire baskets instead of in battery jars. One hundred and thirty-six lesions were examined. Infection appeared to have occurred through the uninjured skin in 6 cases. There were definite abrasions or disturbances in the surface tissue of 119 lesions. Infection occurred through rootlets in the remaining 11 cases.

The results of these experiments indicate that infection may sometimes take place through the uninjured skin. It would seem that

as a rule the pathogene penetrates the tissue of the host where the skin offers the least resistance, although, of course, it is possible that the slight abrasions or defects in the skin may have been developed as a result of the action of the fungus.

TIME REQUIRED FOR INFECTION

Infection had occurred at temperatures of 23°, 24°, and 27°C. in 4 days; at 31°, 28°, and 20° in 5 days; at 18.5° in 6 days; and at 14° in 8 days. Judging by the size of the lesions, infection occurred at the various temperatures in the following order: 25° (3.4 mm. diameter), 28° (2.6 mm.), 23° (2.1 mm.), 31° (1.9 mm.), 20° (1.4 mm.). These measurements are the average diameters of 5 spots in each case at the end of 5 days. The average diameter of 5 lesions after 8 days at 14° was 1.4 mm. and at 18° 3.3 mm. The time required for infection to become evident varied from 4 to 8 days over a range of temperatures from 14° to 31°.

INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF THE DISEASE

Various methods were employed to measure the influence of temperature on the development of disease. In the first experiments the potatoes were inoculated by dipping in a spore suspension. They were then maintained at various temperatures for a period of 21 days. After 13 and 21 days the diameters of the lesions were measured by means of a caliper. Two methods of estimating the quantity of decay present at the various temperatures were employed. First, the diameter of the lesions was measured, and second, the area of the lesions was determined. The data were computed from two experiments by selecting the 10 largest lesions at each temperature in each experiment and striking an average of the 20 lesions. The area was determined by computing the area of each lesion and striking an average of the 20 areas. The use of the largest lesions that develop at each temperature as a standard of measurement is open to criticism, because in some instances (at the border temperatures) the number of lesions may have been too small for comparison with those where there was a larger number to select from. Moreover, by this method the amount of decay that occurs at all temperatures is exaggerated. However, the general relations found are fairly comparable to those obtained by other methods.

TABLE 2.—*Influence of temperature on infection of sweet potato by black rot and development of the disease after 13 and 21 days*

Temperature	Diameter of lesions after—		Area of lesions after—	
	13 days	21 days	13 days	21 days
° C.	Mm.	Mm.	Sq. mm.	Sq. mm.
34.4	0	0	0	0
32.0	4.4	7.6	15	48
29.0	10.0	17.1	82	232
24.3	11.2	17.8	98	254
23.0	11.2	16.5	100	211
20.0	6.6	11.2	36	99
18.0	6.8	10.8	34	92
14.3	2.6	5.0	5	20
11.6	0	0	0	0

The data for these experiments are recorded in Table 2 and are graphically illustrated in Figure 2. The minimum temperature for infection for the time employed lies between 11.6° and 14.3° C., the maximum between 32° and 34.4° , and the optimum between 23° and 29° .

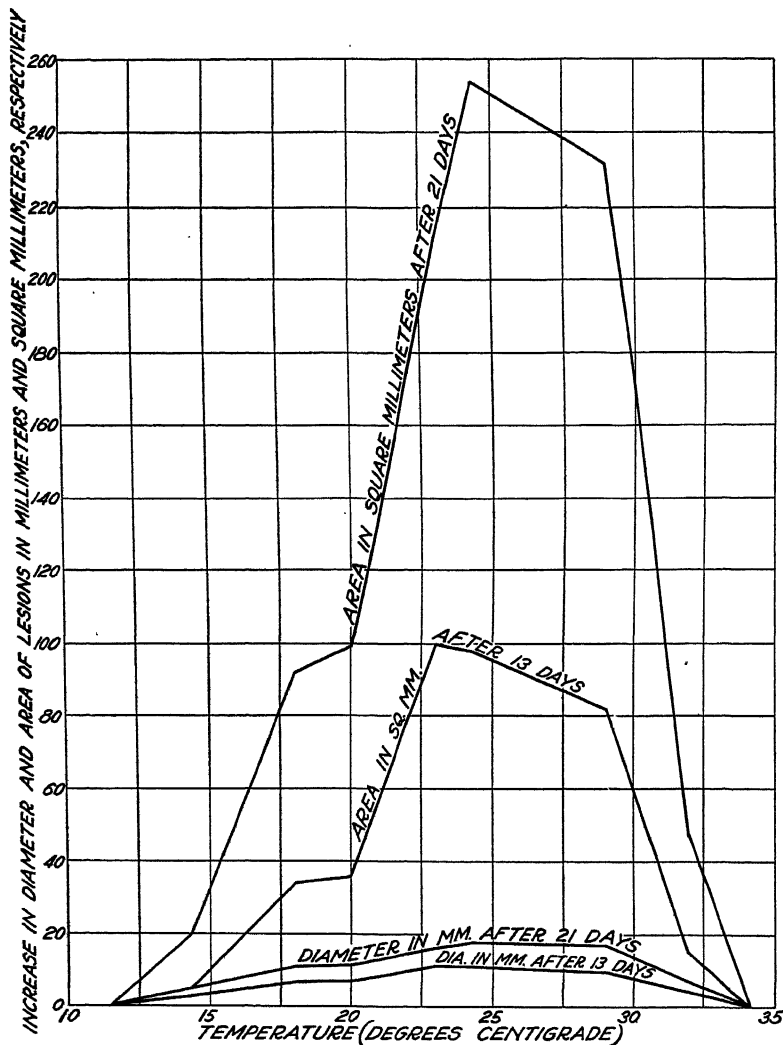


FIG. 2.—Curves representing the enlargement of lesions caused by *Ceratostomella fimbriatum* after 13 and 21 days

The effect of the passage of time was to enlarge the scope of the decay, except at 11.6° and 34.4° C., where there was none. More time would have allowed the limits of the infected areas to widen slightly. The difference in the amount of decay after the two periods of time (13 and 21 days) becomes progressively larger as the temperatures change from the maximum and minimum to the optimum. This relation is well illustrated by the area curves.

The diameter of fungus colonies and of fungus lesions have been used by other writers as a measure of the growth phenomena involved in the two cases. Although this standard affords a means of comparing the effect of different temperatures, it does not show the true relation of temperature either to the growth of the fungus or the decay involved in the enlargement of lesions of this type. The area of the lesions comes more nearly representing the actual quantity of decay (where the latter is a surface phenomenon) at a given temperature, and therefore the relation of decay to the different temperatures. The difference between the diameter curves and the area curves is striking (fig. 2); so striking, in fact, that one sees no good reason for using diameter as a measure of the amount of decay.

In the preceding experiments a comparison was made of the large lesions only that developed at the different temperatures. It seemed probable that there would be considerable variation in the rate of development in the various lesions, and consequently that a different relation might be found if the total number of lesions that developed at the various temperatures were used as a basis of comparison.

TABLE 3.—*Influence of temperature on infection of the sweet potato by black rot and on enlargement of the lesions after 11 days*

Psychrometer readings	Depression of wet bulb	Average area of lesions	Number of lesions
° C.	° C.	Sq. mm.	
36.0-34.6	1.4	0	0
33.0-31.8	1.2	0	0
31.5-28.9	2.6	16	3
29.5-27.8	1.7	31	32
27.0-25.0	2.0	73	35
24.0-23.0	1.0	58	74
23.0-22.0	1.0	49	163
20.0-19.1	0.9	27	165
18.5-18.0	.5	20	152
14.0-13.6	.4	7	22
11.8-11.0	.3	0	0
9.5- 9.3	.2	0	0
8.0- 7.8	.2	0	0

Table 3 gives the results of an experiment where 20 potatoes were inoculated in a spore suspension and placed at each of several temperatures. After 11 days all the lesions that had developed were measured. It will be noted that the number of lesions is somewhat smaller at the border temperatures than at the intervening temperatures. How greatly this fact influences the total area of lesions at a given temperature can not be stated with certainty, but judging from the curves obtained it seems improbable that the effect is large (fig. 3). There seems to be some correlation between the humidity of the air and the number of lesions, although the difference in the number of lesions at 24° and 23° C. is rather large, notwithstanding the fact that the depression of the wet bulb is the same at both temperatures.

A smoother curve (fig. 3) was obtained by this method than by that previously used. It is believed that this curve shows more accurately the decay present at the various temperatures than do the area curves in Figure 2, because it represents all of the lesions rather than a selected few. No breaks occur in it, and the cardinal temperatures are more definite. The results shown in Figure 3 were confirmed by another experiment which was run for 13 days.

INFLUENCE OF TIME ON THE DEVELOPMENT OF THE DISEASE

Some data were presented in Table 2 to show the correlation of time and size of lesions at various temperatures. These data permit of a comparison of the combined process of infection and enlargement of the lesions at one temperature with that at another temperature, but do not permit of a comparison of the enlargement of the lesions alone at different temperatures. The data in Table 4 show the influence of temperature after different periods of time on the enlargement of lesions as measured by their increase in area.

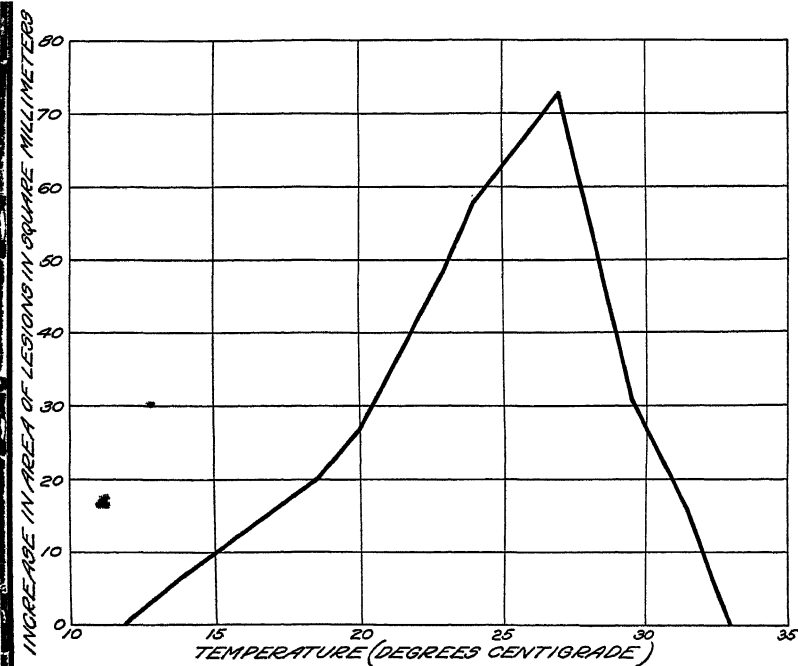


FIG. 3.—Influence of temperature on development of black rot in the roots of sweet potatoes

TABLE 4.—Influence of temperature on the enlargement of selected lesions in sweet potatoes after different periods of time

Tem- pera- ture	Average area of lesions in square milli- meters after—						
	8 days	11 days	14 days	18 days	22.5 days	28 days	64 days
° C.							
33.0	12	23	23	24	27	32	----
31.0	12	26	32	37	46	-----	-----
28.0	11	27	32	42	60	109	-----
25.0	11	24	31	40	52	119	-----
23.0	13	29	39	51	69	115	-----
20.0	11	20	27	35	49	69	-----
18.5	12	25	35	49	60	139	-----
14.0	12	22	26	30	36	60	-----
12.0	13	18	19	21	26	29	-----
9.5	11	15	20	-----	23	-----	-----
6.0	13	16	16	-----	19	-----	20

The data were derived from two experiments and the averages were computed from 15 lesions at each temperature, except after 28 and 64 days, where the averages were computed from 7 lesions at each temperature and were obtained from one experiment. The potatoes were inoculated by being dipped in a water spore suspension. They were then placed at a temperature of 23° C. for a period of 5 days to permit infection to take place. The lesions were then measured and numbered, so that they could be recognized for subsequent measure-

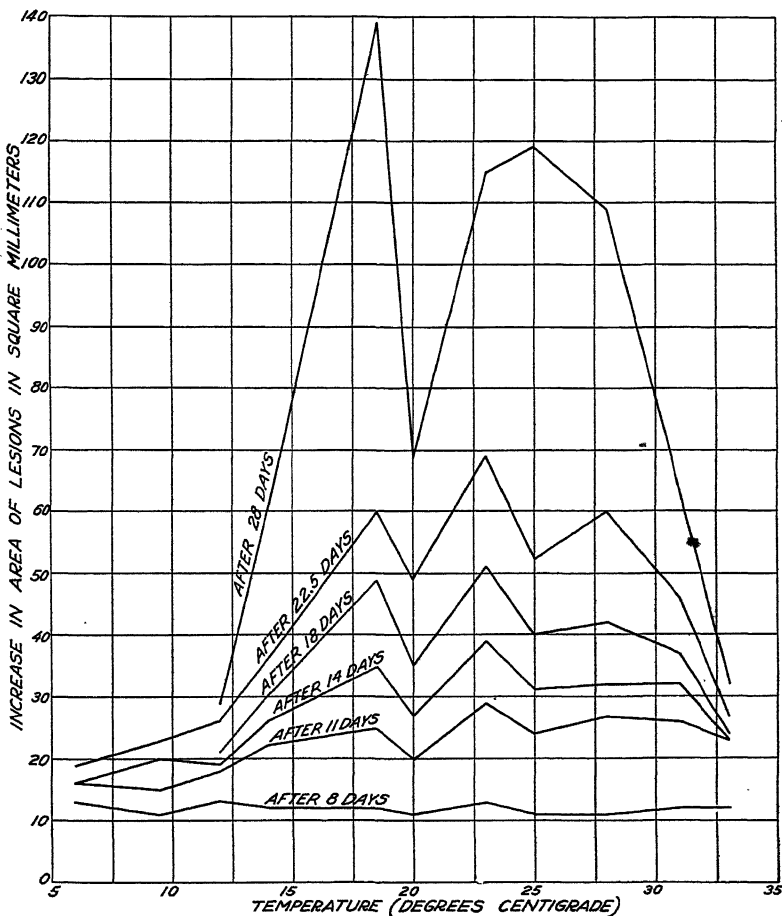


FIG. 4.—Enlargement of lesions in sweet potatoes after different periods of time

ments at different intervals of time. As soon as this was done the potatoes were divided into lots and distributed to the various temperatures in such a way that the sizes of the lesions were fairly comparable at the different temperatures. It will be seen by examining the curve labeled "After 8 days" in Figure 4 that the line is fairly straight. There are some variations in it, most of which persist throughout the successive periods. This fact indicates the difficulty of comparing directly the process involved in the enlargement where the areas of action are not identical from the beginning. These

variations are in some instances overcome at the more favorable temperatures and in some instances accentuated. With the exceptions noted, the curves (fig. 4) plotted from the data obtained in these experiments generally tend toward the normal curve with the lapse of time. These exceptions may be partially explained, at least. An examination of the notes from which these data were derived reveals a considerable variation in the rate of growth of the different lesions at a given temperature, and in some instances shows that the lesions stop enlarging altogether at different stages of development. These factors, especially the latter, are shown to be correlated with the variations in the curves. If the curves had been plotted from a larger number of lesions, these variations probably would not have been so evident.

There was some enlargement at all the temperatures employed (Table 4). It was not large at 33° and 12° C. and below. The enlargement at these extreme temperatures will be discussed more fully under the heading "Temperature limits of infection."

INFLUENCE OF TEMPERATURE AND TIME ON THE NUMBER OF INFECTIONS

The data recorded in Table 3 showed that temperature was a factor in determining the number of infections which occurred during the time employed (11 days). The number of infections rose from zero at 11.8° C. to 165 at 20°, but dropped off rapidly as the temperature rose from 23° to 31.5°, where there were only three infections; at 33° there were none. Humidity may have influenced this drop in the number of infections, for there was a general decrease in the relative humidity with the rise in temperature above 24°. However, this factor does not account for the difference in the number of infections at 23° and 24°, where the humidity was identical. Unpublished data show that there is a narrow range of humidity just below saturation, at a temperature of 23°, within which the number of infections is little affected by the difference in humidity, but that the number of infections drops rapidly after the humidity falls below this range. It can not be said definitely that this relation holds at other temperatures, but it would seem likely, and it is possible that the limit of this range of humidities was reached at 27° and 31.5° (Table 3).

TABLE 5.—Influence of temperature on the number of lesions that develop after 21 days

Psychrometer readings		Relative humidity	Number of sweet potatoes	Number of lesions per potato
Temperature	Depression of wet bulb			
° C.	° C.			
33.4			18	0.3
30.8	1.2	92	24	9.5
28.2	1.2	92	19	14.3
23.6	.9	93	24	19.4
23.0	1.0	92	20	16.1
19.9	1.4	88	24	13.4
18.3	.9	92	24	11.8
14.3	.8	91	18	21.0
12.0	.3	97	16	.06
9.0	.9	90	17	.0

The data recorded in Table 5 were obtained from an experiment designed to determine whether or not temperature governs the number of infections to the same degree if a longer period of time is em-

ployed. The results showed that the border temperatures limit the number of infections, but the increase in the number of infections from the minimum to 23.6° C. (near the optimum) was much smaller, and the maximum number of infections was obtained at 14°, which is several degrees below the optimum temperature for the development of decay. This exception would seem to be due to experimental error. One might account for the small difference in the number of infections in the same way at 18.3°, 19.9°, 23°, and 23.6° were it not for the direct correlation which exists between the number of infections and the rise in temperature within this range. An inverse correlation is also apparent between the number of infections and the depression of the wet bulb, the depression of the wet bulb decreasing from 1.4° at 19.9° to 0.9° at 23.6°.

Thus humidity may partially explain the difference in the number of infections at these temperatures. In any case, it is believed safe to conclude from the data presented that temperature as a determining factor in the number of infections becomes less important with the lapse of time, except at the border temperatures. This conclusion is confirmed by earlier work.⁷

TABLE 6.—*Temperature limits at which Ceratostomella fimbriatum infects sweet potato*

Psychrometer readings		Inoculated sweet potatoes						Remarks	Checks		Duration of experiment
Temperature	Depression of wet bulb	Sweet potatoes used	Sweet potatoes infected	Total lesions	Diameter of lesions	Isolations made	Organisms isolated		Sweet potatoes used	Sweet potatoes infected	
° C.	° C.	No.	No.	No.	Mm.	No.			No.	No.	Days
33.4	1.3	16	4	5		0					21
34.7	1.3	45	0	0	0	8	Fusarium sp.	8 doubtful lesions.			62
11.7	.5	16	16	153	3 to 18	0					62
11.0	.6	31	30	240	3 to 13	0					62
9.7	.6	56	8	1	4			8 doubtful lesions.			62
13.4	.4	14	13	78	3 to 15	0			13	0	56
11.1	.2	17	8	26	3 to 10	0			10	0	56
9.1	.2	11	0	0	0	0			9	0	56
13.2	1.1	15	3	12		0			15	0	103
11.4	.8	16	2	3		3	Ceratostomella fimbriatum.				103
9.5	.6	14	5	10		5	3 Fusarium sp.; 1 Alternaria sp.; 1 Penicillium sp.				103
13.2	1.0	18	17	99	5 to 50	0			18	0	108
11.5	.9	17	16	101	5 to 20	6	Ceratostomella fimbriatum.				108
9.5	.6	18	2	2	5 to 8	4	2 Fusarium sp.; 2 Penicillium sp.	1 doubtful lesion.			108

TEMPERATURE LIMITS OF INFECTION

The data recorded in Table 6 were obtained from three experiments, which had for their purpose the determination of the upper and lower temperature limits for infection. The potatoes were inoculated by dipping in a spore suspension. In one of the experiments isolations were made from the lesions that developed at the border temperatures. This procedure was adopted because in some instances the lesions were not typical. Checks were run in some cases to make sure that the infections were due to the inoculum. The check potatoes were dipped in tap water instead of a spore suspension.

⁷ AMES, A. THE TEMPERATURE RELATIONS OF SOME FUNGI CAUSING STORAGE ROTS. *Phytopathology* 5: 11-19. 1915.

Five typical black-rot lesions developed at a temperature of 33.4° C. in 16 days. This temperature is almost as high as the highest temperature (34.5°) at which *Ceratostomella fimbriatum* grew on sweet-potato agar (Table 1). There were no infections at 34.7°, even at the end of 62 days. It would seem, therefore, that the maximum temperature for infection was between 33.4° and 34.7° C.

The lowest temperature at which infection occurred was 9.5° C. This conclusion is based entirely on the character of the lesions, which, however, seemed to be typical. *Ceratostomella fimbriatum* was not obtained in any of the isolations made at this temperature. It would seem that if any infection did occur the pathogene either died out or gave way to other fungi. At any rate the infection that occurs at this temperature is of little consequence. This contention is further supported by the fact that no growth of the pathogene, except in one doubtful case, occurred at 9.5° C. in 55 days and very slight growth occurred at 10° in 73 days. No infection occurred at 9.1° in 56 days. Infection took place fairly readily at 11° and the pathogene was recovered at 11.4°. It would seem from these results that the lower temperature limit for infection lies between 9° and 11°.

These results indicate that sweet potatoes can be stored at temperatures from 9° to 10° C. without much danger of infection, although the pathogene may be present. A humidity below 90 per cent would further insure against infection.

If infection has already occurred some enlargement of the lesions may be expected at 9.5° C. (Table 4) and possibly at even a lower temperature, but it would be too slight to be of any consequence. Even at 12° the enlargement of lesions is rather slow.

The slight enlargement of the lesions at temperatures below the minimum may have been influenced by the removal of the potatoes to the laboratory (the temperature of the laboratory ranged from 20° to 23° C.) during the periods in which the measurements were made. The time consumed by these measurements was never more than a half hour, except when the first measurements were made; hence it is believed that this change of condition had little effect on the enlargement of the lesions.

The temperatures, therefore, that recommend themselves for the storage of sweet potatoes where black rot is a factor are those from 10° to 12° C., since sweet potatoes can not be stored below these temperatures without becoming infected with *Mucor racemosus*.⁸ It is believed possible to practically eliminate the spread and development of black rot if the potatoes are stored at these temperatures and at a relative humidity below 90 per cent.

DISCUSSION AND CONCLUSIONS

Ceratostomella fimbriatum will grow on sweet-potato agar over a range of temperatures extending from 9.5° to 34.5° C. (Table 1). This range corresponds closely to that obtained for infection. However, infection was doubtful at the two extreme temperatures of this range. Growth of the pathogene, as represented by the area of the colonies, increases very rapidly as the temperature rises from the minimum (9° to 10° C.) to 27° after which it declines with the rise in temperature until 36° is reached, at which point all growth ceases.

⁸ LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441-456, illus. 1923.

This rate of growth is due not only to temperature but to the area of the medium to which it is exposed, which progressively increases with the increase in diameter of the lesions.⁹

Infection may occur through the normal skin, but usually there is evidence of tissue disturbances at the point of infection in the form of raised areas, slight abrasions, and small rootlets. Wounding is not necessary in order for infection to occur, although there is evidence that wounding will aggravate infection and the development of the disease.

The increase in the size of the lesions with the lapse of time at any of the temperatures between the maximum and the minimum is not as rapid as the enlargement of the colonies of the pathogene on sweet-potato agar. This fact would seem to indicate that either sweet-potato agar is the more favorable medium for the development of the fungus or that there is some inhibiting factor retarding the enlargement of the lesions.

The fact that the lesions enlarge at temperatures below the minimum for infection (Table 4) while the fungus does not grow at these temperatures may raise some question as to the foregoing conclusion, at least under the latter conditions indicated. What would happen if growing colonies of the fungus on artificial media were exposed to these temperatures is not known, but it seems probable that the colonies would enlarge as did the lesions. It has been observed that new transfers of fungi do not exhibit the normal rate of growth at the start. To make the above conclusion valid more experimentation is required.

The rate of enlargement as represented by the area of the lesions increases rapidly as the temperature rises above the minimum (9° to 10° C.) to temperatures of 23° and 27° C., above which there is a decline in the rate as the temperature is raised to 34.7°, where there is no infection.

A part of this increase¹⁰ in the rate of enlargement of the lesions with the rise in temperature is due to the increase in the area exposed to the action of the fungus with the increase in the diameter of the lesions. This increased area of action of the fungus as the temperature is raised to the optimum becomes progressively greater with the lapse of time, thus enhancing the enlargement of the lesions.

Taking into consideration the methods employed in measuring the influence of temperature on the development of the disease, it may be concluded that the optimum ranges from 23° to 27° C. This variation is not large when it is considered that three methods of procedure and several experiments were employed. Even where the same method was used in two different experiments some variation occurred. This was shown by the fact that in an experiment conducted in the same fashion as the one from which the data recorded in Table 3 were obtained the optimum was 24.5°, while the optimum recorded in Table 3 is 27°. It is believed that the optimum for the growth of a fungus, for infection, or for the development of the disease can never be assigned to a particular degree of temperature. The variation in the fungus and the host, even though the conditions surrounding them may be constant, is such as to make the optimum limited to a particular temperature improbable except in a single experiment.

⁹ LAURITZEN, J. I., and HARTER, L. L. THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND BEGAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS. Jour. Agr. Research 30: 793-810, illus. 1925.

¹⁰ LAURITZEN, J. I., and HARTER, L. L. Op. cit.

The range of temperature at which black rot will develop is wider than that at which infection will occur. Infection has taken place at temperatures of 9.5° and 33.4° C. (Table 6), while there has been an enlargement of lesions at temperatures from 6° to 33.4° (Tables 4 and 6). This fact has a practical bearing. Potatoes that have not been infected when they are placed in storage, although the pathogene is present, can be kept at temperatures of 10° to 12° without much danger of infection, especially if the relative humidity is below 90 per cent. If infection has taken place, the lesion will continue to develop (Table 4, fig. 4). Fortunately the rate of enlargement is very slow, hence the disease can be held in check. Besides there is some evidence that the fungus dies out at a temperature of 9.5° (Table 6). Since potatoes that are infected are not regarded as edible, the holding of the disease in check loses some of its significance. Temperature governs the number of lesions during the early stages of infection (Table 3), but as the time is extended from 11 to 21 days its influence becomes strikingly less at temperatures between 14° and 28° C. (Table 5), where there is little difference in the number of infections. The number drops off below and above these temperatures.

SUMMARY

The temperature range for the growth of *Ceratostomella fimbriatum* on sweet-potato agar extends from 10° to 34.5° C.

The optimum, maximum, and minimum temperatures for the growth of the fungus are 23° to 28.5° C., 34.5° to 36°, and 9° to 10°, respectively.

The range of temperatures at which *Ceratostomella fimbriatum* will infect sweet potatoes extends from 9.5° to 34.5° C., corresponding closely to that of the growth of the pathogene. The fungus seems to die out at a temperature of 9.5°.

The optimum, maximum, and minimum temperatures for infection and the development of the disease are: Optimum, 23° to 27° C.; maximum, 34.5° to 36°; and minimum, 9° to 10°.

The number of infections that will occur between 9° and 11° C. is small.

The temperature range at which it has been shown that black-rot lesions will enlarge is from 6° to 33.5° C.

The rate of enlargement is slow between 6° and 14° C. and increases very rapidly above 14° until it reaches a maximum between 23° and 27°. It varies greatly at any given temperature and in some instances ceases altogether.

The number of infections is governed by the temperature which prevails (Table 3) during the early stages of infection (11 days). The number of infections increased from 22 at 14° C., the minimum for this period, to a maximum at temperatures near the optimum for the development of the disease (20° and 23°). With a further rise in temperature the number of infections decreased to three at 31.5° and at 33° ceased altogether.

As the experimental period was extended from 11 to 21 days (Table 5) the influence of temperature on the number of infections became less apparent, except at the border temperatures. The differences in the number of infections at temperatures of 14°, 18°, 20°, 23°, 23.6° and 28° are relatively small.

FRUIT-BUD DIFFERENTIATION AND SUBSEQUENT DEVELOPMENT OF THE FLOWERS IN THE HICORIA PECAN¹

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INTRODUCTION

A correct understanding of the time of fruit-bud differentiation and some of the related activities of the pecan should have an important bearing upon cultural practices. More efficient methods of fertilizing and pruning pecan trees might be evolved from accumulated information of this kind.

The purpose of the present study is to determine the time at which flowers are differentiated and to trace their development to the time of pollination. The question resolves itself into a study of the changes that take place in bud development, the time when these changes occur, and the conditions that influence them.

Benson and Welsford³ described the morphology of the ovule and female flowers of *Juglans regia*, with special reference to the vascular system. Holm⁴ described the staminate flower of *Carya alba* and of *Juglans nigra*. Stuckey⁵ studied certain phases in the development of the pecan flowers. He recorded, for more than 25 varieties of *Hicoria pecan*, the dates on which the staminate flowers reach maturity and shed their pollen and the dates on which the pistillate flowers become receptive.

The senior author,⁶ in a study of the development of the staminate flowers of 28 varieties of pecan, found that catkins are differentiated in lateral buds on new shoots in late spring. They pass the winter as dormant buds and complete their development the following spring.

METHODS

Terminal buds of the Alley and Frotcher varieties were collected every other day, beginning March 13, 1925, and continuing until the pistillate flowers were separable as individual flowers on April 7 and 11, respectively. Flowers were then collected every second day until pollination time. The following season buds were collected at 20-day intervals from November until March. The Stuart and Jerome varieties were extensively used in detailed studies of the flower at pollination time.

Material was fixed in both Flemming's weaker solution⁷ and Juel's fixative.⁸ Flemming's solution rendered the material too brittle to

¹ Received for publication Apr. 20, 1926; issued October, 1926.

² The writers are indebted to B. B. Higgins of this station for constructive criticisms of the technic employed in the investigations herein reported.

³ BENSON, M., and WELSFORD, E. J. THE MORPHOLOGY OF THE OVULE AND FEMALE FLOWER OF JUGLANS REGIA AND OF A FEW ALLIED GENERA. *Ann. Bot. [London]* 23: 623-633, illus. 1909.

⁴ HOLM, T. MORPHOLOGICAL STUDY OF CARYA ALBA AND JUGLANS NIGRA. *Bot. Gaz.* 72: 375-389, illus. 1921.

⁵ STUCKEY, H. P. THE TWO GROUPS OF VARIETIES OF THE HICORIA PECAN AND THEIR RELATION TO SELF-STERILITY. *Ga. Agr. Expt. Sta. Bul.* 124: 127-148, illus. 1916.

⁶ WOODROOF, J. G. THE DEVELOPMENT OF PECAN BUDS AND THE QUANTITATIVE PRODUCTION OF POLLEN. *Ga. Agr. Expt. Sta. Bul.* 144: 134-161, illus. 1924.

⁷ CHAMBERLAIN, C. J. METHODS IN PLANT HISTOLOGY. Ed. 3, rev. 314 p., illus. Chicago, Ill. [1915.]

⁸ Formula for Juel's fixative: $ZnCl_2$, 2 gms; acetic acid, 2 c. c.; 95 per cent alcohol, 50 c. c.; distilled water, 50 c. c.

section well. Juel's fixative proved more satisfactory and was used for most of the work. The buds were somewhat difficult to manage owing to the heavy growth of hairs on the young leaves. It was necessary to trim them very closely so as to remove as many as possible of the young leaves.

All material was embedded in paraffin and the sections stained in Haidenhain's iron-alum haematoxylin.

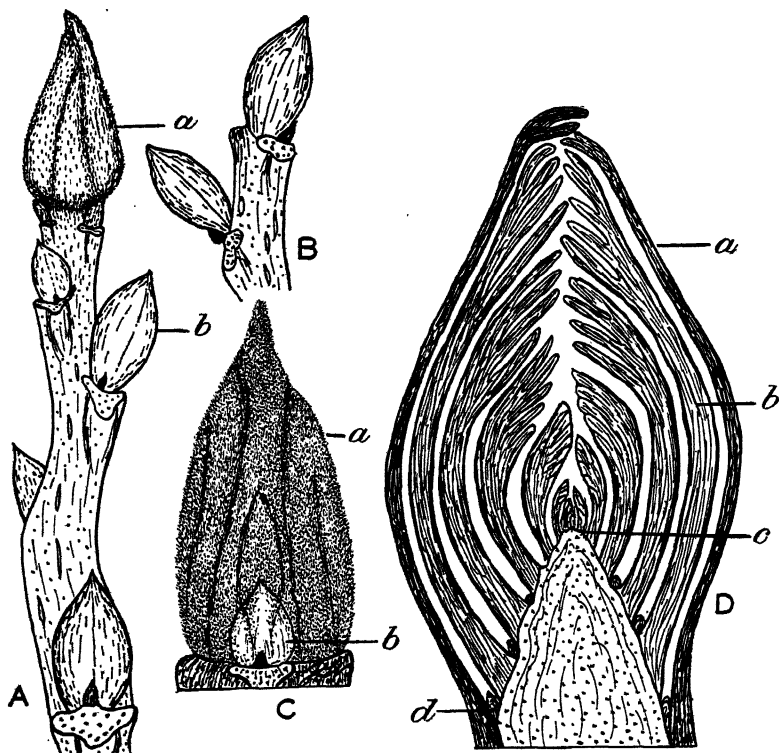


FIG. 1.—Types and position of buds. A, shoot bearing terminal (a) and lateral (b) buds; B, shoot with only lateral buds; C, terminal bud after the scales are shed (a) young leaf, (b) lateral bud; D, internal structure of terminal bud; (a) terminal bud scale; (b) inclosed leaves; (c) point of differentiation; (d) lateral bud

DIFFERENTIATION IN THE TERMINAL BUDS

The pistillate flowers are normally differentiated from the terminal buds of the dormant season, but when a terminal bud is not present one or more lateral buds may differentiate pistillate flowers.

BUDS

In the absence of a terminal bud the casual observer will often mistake the apical, dormant, lateral bud for a terminal bud, since the scar left by the shedding of the nut cluster of the previous season is very small and inconspicuous. The color of the two buds is similar, but the terminal buds (fig. 1, A, a) are angular, uneven, and somewhat larger than the dormant lateral buds (fig. 1, A, b).

The terminal buds do not have the same type of scales as the lateral buds, but are protected by the thick, flattened, outer leaves, which are practically identical with the inner dormant leaves of the bud. Several of these leaflike scales are shed as the bud swells and only the innermost develop into leaves. In the axil of each scale is a very small lateral bud (fig. 1, D, *d*).

LEAVES

A terminal bud in late winter contains the full number of leaves that will develop the following season. This number is definitely determined when the first pistillate flower appears. The larger leaves grow over and around the smaller and more recently formed ones (fig. 1, D). In the axil of each scale and leaf is a developing lateral bud of the following season. (Fig. 1, D, *d*.) The young leaves are light green in color, covered by a thick growth of silvery hair.

FLOWERS

Woodroof⁹ concluded that the internal character of the buds does not materially change from the time of harvest in November until the middle of February (fig. 2, A), and that differentiation of the first pistillate flowers occurs during the last 10 days of February and the first week in March.

By the second week in March some of the flowers are becoming quite large and the floral characters are beginning to appear (Table 1). The basal flowers appear first, followed by others placed alternately along the elongating floral axis (fig. 2, B and C). Several stages of development may be seen in a differentiating cluster of flowers (fig. 2), and this is true even when most of the flowers of the cluster are ready to be pollinated. The basal flowers show some of the floral characters while the apical ones are mere papillae and others have not been differentiated. Simultaneously with the differentiation and early development of the flowers, the scales are shed and the leaves begin to unfold.

Where the terminal bud has been present, but was destroyed before the time of differentiation (from February 15 to March 1), lateral

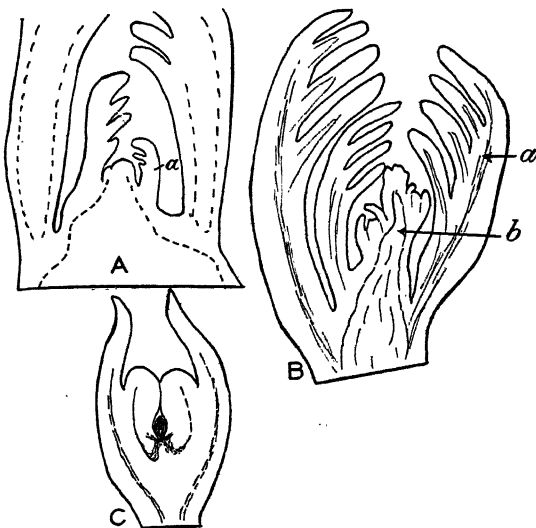


FIG. 2.—Pistillate flower differentiation. A, growing point of a terminal bud during the winter; B, growing point on March 13, after several pistillate flowers (*b*) have been differentiated; *a*, subtending leaves; C, young basal flowers on date when first flower clusters were visible above the leaves. Terminal drops on May 1 have reached a similar stage of development. All $\times 9$.

⁹ WOODROOF, J. G. THE DEVELOPMENT OF PECAN BUDS AND THE QUANTITATIVE PRODUCTION OF POLLEN. Ga. Agr. Expt. Sta. Bul. 144: 134-161, illus. 1924.

buds are "forced" to differentiate pistillate flowers; but if destroyed later than March 15-20 there will be no pistillate flowers to reach the receptive stage, and a crop failure for that year will result. In pecan trees which bear an annual crop of nuts a large percentage of the clusters of pistillate flowers must come from twigs produced from lateral buds (fig. 3). Theoretically, the pecan is strictly an alternate year bearer; while practically it may bear annual crops because the years in which shoots from terminal buds produce nuts, shoots from lateral buds do not bear, but produce terminal buds in preparation for a crop the following year.

TABLE 1.—*Stages of development in the buds and flowers of the pecan and the dates on which they occur*

Date	Pistillate flowers or buds	Staminate flowers or buds	Vegetative growth ^a
Feb. 15 to Mar. 1...	Differentiation in terminal buds.	Outer bud scale bursting....	Very little.
Mar. 11 to Mar. 15...	Bud scales bursting; differentiation continued.	Shedding of outer bud scale.	Do.
Mar. 15 to Apr. 1...	Differentiation of apical flowers; development of basal flowers.	Outer scale shedding completed.	Very young leaves exposed.
Apr. 1 to Apr. 15...	Rapid development; may appear visible above leaves.	Inner bud scale bursting....	Exposed leaves growing rapidly.
Apr. 15 to May 1...	Flowers reach full size; may become receptive.	Catkins reach full size; may shed pollen.	Very rapid.
May 1 to May 15...	Pollination completed; stigmas dry; first "drop."	Pollen shed; catkins drop; flowers may be differentiated in new buds.	Do.
May 15 to June 1...	Rapid growth of nutlets....	Rapid development of lateral buds; differentiation of flowers.	Do.
June 1 to Oct. 1....	Continued growth and development of nuts.	Continued development of lateral buds.	Continual growth.
Oct. 1 to Nov. 1....	Nuts mature.....	Lateral bud growth decreases.	Terminal bud may form. ^b
Nov. 1 to Feb. 1....	None.....	Very slow growth in lateral buds.	Almost inactive.

^a Though the formation and development of leaves has not been systematically studied, careful observation has shown that differentiation is active in the vegetative portion of the lateral buds as well as in the terminal bud during the late fall and winter. In the lateral buds the process continues throughout the spring and summer into the fall and winter until the terminal bud differentiates pistillate flowers in February of the next year. Leaf differentiation ceases on a particular shoot when pistillate flower differentiation begins.

^b If the shoot was terminated with a cluster of nuts during the current year no terminal bud will be formed. A scar will mark the place of attachment of the nut cluster. Almost immediately upon the formation of the leaf a lateral bud forms in the axil, in which is differentiated staminate flowers. Thus staminate flower formation continues over almost the entire growing season.

EARLY DEVELOPMENT OF THE PISTILLATE FLOWERS

The four bracts of the calyx are the first parts of the flower to appear, and these are followed by the primordia of the stigmas. Early in the development of the bracts and the stigmas the traces of a vascular system may be seen in the longitudinal sections of the flower (fig. 2). The stigmas soon inclose the ovarian cavity. An ovule begins to develop in the basal flower about April 15, the approximate date on which the young flower cluster is first visible above the unfolding leaves (fig. 2, C). A week or 10 days after the appearance of the ovule an integument begins to develop.

THE PISTILLATE FLOWER CLUSTER AT POLLINATION TIME

The pistillate flowers develop very rapidly, and are receptive about two months after the differentiation of the basal flowers. The flowers are borne sessilely along a zigzag central axis. There are

from 4 to 20 or more flowers in a single spike. The number of flowers per spike, or flower cluster, is a varietal character. In 1925 about 90 per cent of the Teche and Alley varieties bore 4 receptive flowers per cluster, with a few of 3 and 5 flowers and none with 6. Most of the clusters of the Nelson and Mobile varieties bore 6 receptive flowers per cluster, with a few of 5 and 7, but none with 3 and 4. With the Stuart and Frotcher varieties, 5 was the commonest number.

"DROPS"

Regardless of the number of flowers receptive per cluster at the time of pollination, there are always some undeveloped flowers at the apex of each cluster. (Fig. 4, *a*.) There may be from three to half a dozen of these which differentiate very late but grow and develop normally until the time of pollination, after which they begin to drop without becoming receptive. This first "drop" leaves a scar at the terminus. In no case has a cluster of nuts been observed which had developed to its fullest capacity without showing the small scar indicating the "dropping" of the terminal flowers. Longitudinal sections of these undeveloped flowers show them to be normal, but not so far advanced in development as the other flowers of the cluster. (Fig. 2, C.) In other words, all flowers which are not receptive at the time of pollination are soon shed from the cluster. This first "drop" means that from 20 to 40 and sometimes 50 per cent of the differentiated flowers of a spike are shed at pollination time.



FIG. 4.—A cluster of pistillate flowers. *a*, Terminal flowers of the cluster which have never been found to develop; *b*, a flower which develops only under the most favorable conditions, otherwise it "drops;" *c*, a flower which develops into a nut

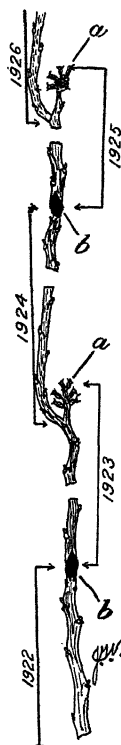


FIG. 3.—The cycle of terminal bud formation: *a*, cluster of nuts produced in "even" years; *b*, terminal bud produced in "odd" years

INDIVIDUAL FLOWERS AT POLLINATION TIME

EXTERNAL CHARACTERS

An individual flower measures from 5.5 mm. to 8 mm. in length, from 3 mm. to 4.5 mm. of which are inclosed in a light green pubescent calyx; the remainder is stigma. The calyx is marked by four ridges, giving it a four-sided appearance and dividing it into four sections, each of which is terminated by a tapering bract from 3

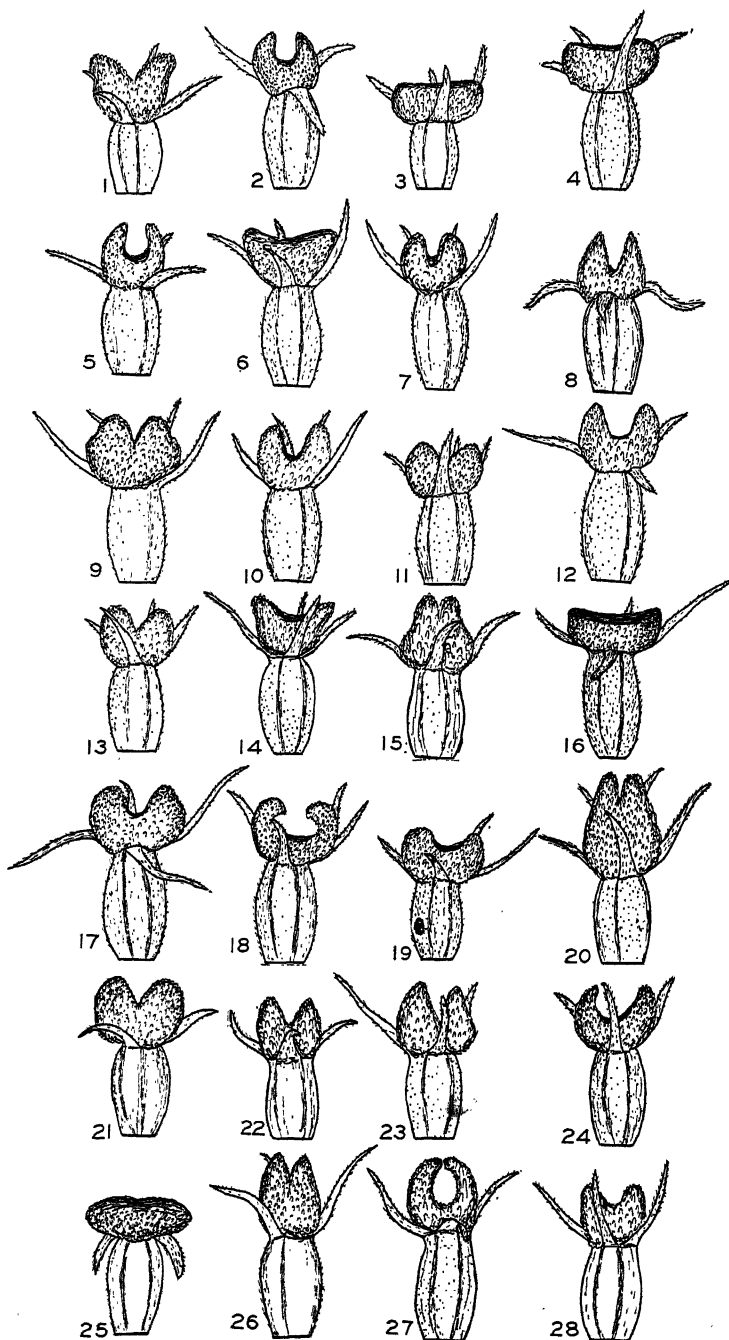


FIG. 5.—Pistillate flowers of 28 varieties of pecans, showing relative size, shape, and curvature of the stigmas, length and curvature of the bracts, and relative size of the entire flower. $\times 4$. (See Table 2 for varieties and colors of stigmas to correspond with the numbers indicated)

mm. to 5 mm. in length, according to the variety. These may be erect, extend laterally, or be reflexed downward along the sides of the flower (fig. 5). Receptive stigmas are sessile, variously shaped, and colored, and a viscid fluid covers the uneven surfaces. The differences among varieties in the size, color, and shape of the stigmas are varietal characters and are very distinct. The writers have not been able to relate any of these floral characters with later characters that appear in the mature nut.

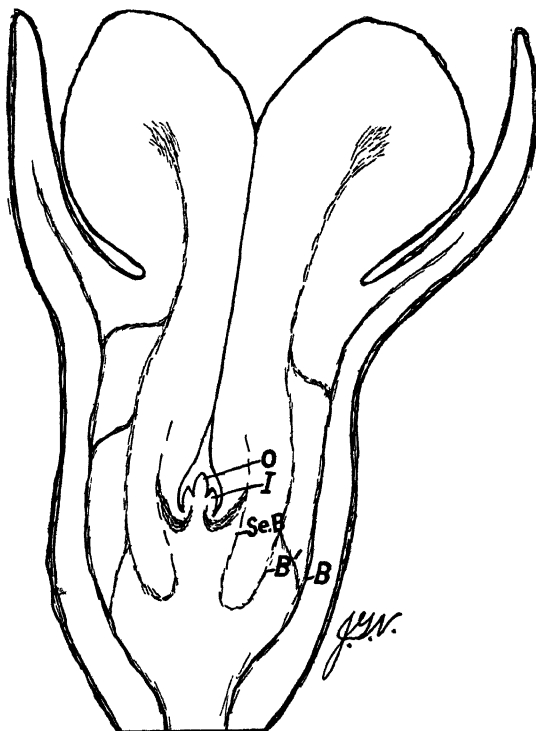


FIG. 6.—Longitudinal section of flower at time of pollination in the plane of the middle septum. O, ovule; I, integument; Se. B, septum bundles; B, outer series of vascular bundles; B', inner series of bundles. $\times 9$

TABLE 2.—Name and color ^a of stigmas of each of the 28 varieties of pecan illustrated in Figure 5, April 29, 1925

No.	Variety	Color	No.	Variety	Color
1	Alley.....	Vivid green.	15	Nelson.....	Vivid green.
2	Appomattox.....	Do.	16	Pabst.....	Do.
3	Atlanta.....	Night green.	17	Randal.....	Ox-blood red.
4	Beverage.....	Vivid green.	18	Russell No. 2.....	Brick red.
5	Bradley.....	Bottle green.	19	Rome.....	Dull dark purple.
6	Centennial.....	Ox-blood red.	20	Russell.....	Night green.
7	Curtis.....	Vivid green.	21	San Saba.....	Warbler green.
8	Delmas.....	Night green.	22	Schley.....	Vivid green.
9	Frotscher.....	Vivid green.	23	Stuart.....	Do.
10	Jerome.....	Dull dark purple.	24	Success.....	Ox-blood red.
11	Mantura.....	Vivid green.	25	Teche.....	Night green.
12	Mobile.....	Ox-blood red.	26	Unknown.....	Do.
13	Moneymaker.....	Vivid green.	27	Van Deman.....	Do.
14	Moore.....	Bottle green.	28	Waukenah.....	Olive green.

^a RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

INTERNAL CHARACTERS

The vegetative regions of the flower resemble those of the young growing nut. The vascular system is fairly complete and serves as a means of locating and distinguishing the different regions, which are in some cases rather indistinct.

The outer is the calyx or hull region and is composed of loosely arranged, irregularly shaped cells. A series of fibro-vascular bundles extend into this region from the main axis of the spike (fig. 6, B). The second, or shell region, is composed of small uniformly shaped, compactly arranged cells. Two opposite suture bundles are located

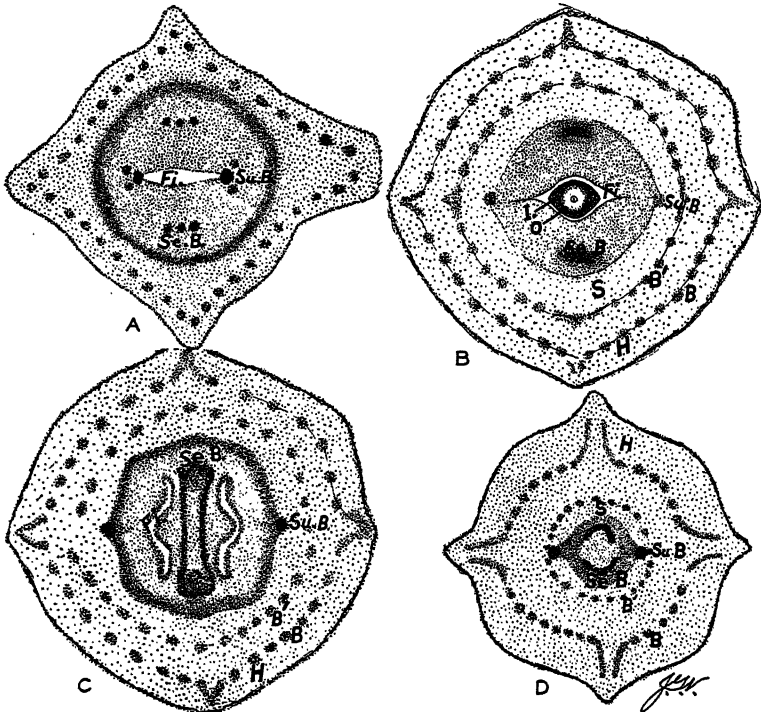


FIG. 7.—Pistillate flower at time of pollination. A, cross section near the base of bracts; B, cross section through the ovule and integument; C, cross section just below the attachment of ovule; D, cross section where the inner series of bundles enter the shell region. H, region of the hull; B, outer series of vascular bundles; B', inner series of bundles; S, region of the shell; Su. B, suture bundles; Se. B, septum bundles; Fi, fissure in packing tissue; I, integument; O, ovule. $\times 9\frac{1}{2}$

in this region at right angles to the plane of the stigmas. These extend upward from the base to the apex of the shell region. Near the apex each separates into three distinct bundles (fig. 7, Su. B).

Between the regions of the shell and hull is a second series of fibrovascular bundles. This series is parallel to the first and is connected with it by lateral branches (fig. 6). The basal extremities of the second series are connected with the vascular system of the shell sutures and of the interior of the flower. The apical extremities extend into the stigma and appear in cross section as a semicircle. The inner series of bundles is not connected with the

outer series at either extremity but only by lateral branches, which are placed at irregular intervals along the sides of the flowers (fig. 6).

The ovary is imperfectly divided by a middle septum located in the plane of the stigmas. The ovule and integument are attached at the base of an opening near the apex of the septum, leaving them free to expand into a fissure (fig. 7, Fi), which is formed in a tissue which almost fills the ovary on either side of the septum. This has been called by Benson and Welsford "packing tissue."¹⁰ It is this fissure which later enlarges to accommodate the expanding embryo (kernel) of the nut.

The ovule is about two-thirds inclosed by the integument when the flowers become receptive (fig. 6), and the vascular system of the septum has become quite distinct. There appears to be no definite communication between the septum bundles and those next the shell region, this not being established until after pollination has taken place. The vascular bundles of the septum develop independently from vascular traces; and later become connected with the series of bundles next the shell region as described by Benson and Welsford¹¹ for *Juglans regia*. Each of the two parallel bundles of the septum become divided near the apex into a semicircle of bundles (fig. 7). Developing fibrovascular bundles are distinguishable in cross sections of the integument.

The embryo sac is not mature at the time of pollination; consequently fertilization can not take place then. Four megaspores appear in nearly all of the ovules on that date, three of which have begun to disintegrate, and the nucleus of the mother cell has divided once and in some cases twice.

SUMMARY

Pistillate flowers in *Hicoria pecan* are differentiated in terminal buds from February 15 to March 1.

In the absence of a terminal bud pistillate flowers may be differentiated in lateral buds.

Pistillate flowers become receptive about two months after differentiation.

The first "drop" occurs at pollination time.

Pistillate flowers are not ready for fertilization at the time of pollination.

Staminate flowers are differentiated in lateral buds from 6 to 12 months before the pollen is shed.

¹⁰ BENSON, M., and WELSFORD, E. J. THE MORPHOLOGY OF THE OVULE AND FEMALE FLOWER OF JUGLANS REGIA AND OF A FEW ALLIED GENERA. ANN. BOT. [London] 23: 623-633, illus. 1909.

¹¹ BENSON, M., and WELSFORD, E. J. OP. CIT.



THE BROWN-POCKET HEART ROT OF STONE-FRUIT TREES CAUSED BY *TRAMETES SUBROSEA* WEIR¹

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INTRODUCTION

The production of prunes and peaches is an important industry in the Pacific Coast States, and any factor which contributes to the lengthening of the productive life of these stone-fruit trees is worthy of consideration. The more the situation is studied the more it becomes apparent that wood decay in this region is the most serious factor which leads to early senility or decrepitude of producing prune and peach trees. The one predominating heart rot of these trees is that produced by *Trametes subrosea* Weir (4).² In a previous paper the writer (6) referred to this fungus as *T. carnea*. The disease of red cedar caused by this organism was described by Von Schrenk (2, pp. 16-21), but its occurrence and destructiveness as an orchard disease has never been described, to the present writer's knowledge.

DISTRIBUTION

In stone-fruit orchards *Trametes subrosea* is found from the coastal and interior valleys of central California northward to the coastal districts of British Columbia. Wherever prunes and peaches are grown in western Washington and western Oregon, this fungus is found doing a major part of the heart-rot damage. In French prune orchards in California where this disease has been serious, this fungus has been responsible for 50 or 60 per cent of the wood decay according to W. W. Thomas, a student of this disease in that State. As is true of all such wood-inhabiting fungi, this disease is most frequently found in orchards which have insufficient care.

Being a wound parasite, *Trametes subrosea* occurs in relation to unprotected infection courts, such as pruning cuts and broken branches. Figure 1 shows the fruiting bodies of the fungus near two unprotected pruning cuts on peach wood.

NATURE OF THE HEART ROT

As the brown cubical rot produced by this organism is more or less restricted to definite pockets in the wood, it has been called brown-pocket rot. Figure 2 shows the decayed pockets in prune wood, in both longitudinal and cross sections, and illustrates the type of this decay.

The individual pockets contain a brown, punky wood which crumbles easily and is usually shrunken and cracked into cubical form.

The changes which the fungus causes in the wood of peach does not differ from that produced in prune. Great chemical changes take

¹ Received for publication Feb. 15, 1926; issued, October, 1926.

² Reference is made by number (italic) to "Literature cited," p. 693.

place in the wood because of the digestive activity of the fungus, and it could be inferred a priori that the group of cytolytic enzymes which give other fungi the power to bring about brown rot must be active agents of *Trametes subrosea*. Nevertheless, the writer carried out some enzymic experiments with the mycelium of this organism grown on peach-wood sawdust, to supplement his observations on



FIG. 1.—Fruiting bodies of *Trametes subrosea* Weir near two unprotected pruning cuts which were improperly made. The host is peach.

the glucoside-splitting and cellulose-hydrolyzing enzymes of *Lenzites saepiaria* (5). After a pure culture of *T. subrosea* had grown on the peach-wood sawdust for about nine months it was removed from the culture jars, dried, and ground. The sawdust was so thoroughly decayed that it was readily ground to a fine powder by rubbing between the palms of the hands. This fungous powder was used in the following experiments.

EMULSIN EXPERIMENT

To test for the presence of emulsin, a 1 per cent solution of amygdalin was used as a substrate, for this is perhaps the most prevalent glucoside in the wood of stone-fruit trees. Fifty cubic centimeter portions of the amygdalin solution were placed in each of two small flasks, and 2 gm. of the fungous powder were added to each. To each of two other flasks 2 gm. of moistened fungous powder were added, and they were autoclaved at 15 pounds for 5 minutes before the 50 c. c. of amygdalin solution were added. In another flask 50 c. c.

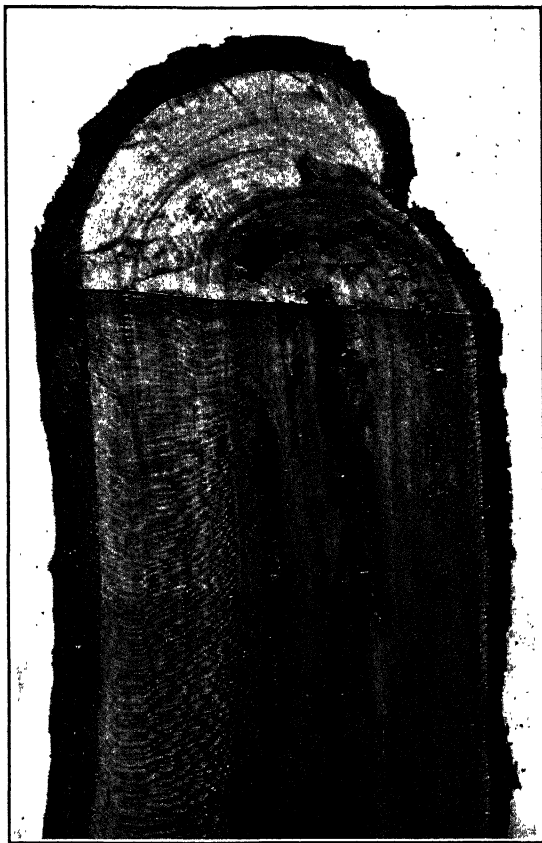


FIG. 2.—Longitudinal and transverse sections of a branch showing the brown-pocket rot in the heartwood. The host is prune

of the amygdalin were placed as a control. To all five flasks sufficient toluene was added for antisepsis. The flasks were corked and allowed to stand at room temperature for five days, after which the two flasks with fungous powder which had not been autoclaved gave a strong odor of benzaldehyde and a very definite Prussian-blue test for hydrocyanic acid. The plain control gave neither of these tests, but the autoclave control gave a slight test for hydrocyanic acid. This is accounted for, perhaps, by the presence of small quantities

of amygdalin in the peach wood used as the culture medium. Thus it was demonstrated that the mycelium of *Trametes subrosea* secretes emulsin as an active digestive ferment.

LIGNINASE EXPERIMENT

A quantity of the cubical brown rot from the pockets produced by *Trametes subrosea* in prune wood was gathered, powdered, and soaked in the least possible amount of chloroformed water for about 24 hours, after which the water was filtered after being expressed from the decayed wood. The enzymes were precipitated with 95 per cent alcohol, collected on filter paper, and dried. This enzymic precipitate was dispersed in a small quantity of water and used in the following experiments.

Very thin shavings of sound prune wood were placed in a flask and digested in a 1 per cent diastase solution for 16 hours. After this they were soaked in several rinsings of distilled water for three days, to remove as much of the soluble substances as possible, and then the shavings were dried.

One gram of these shavings was placed in each of three test tubes. To one was added 10 c. c. of the enzyme dispersion; to another, 10 c. c. of distilled water; and to the third, 10 c. c. of the enzyme dispersion which had been autoclaved at 15 pounds' pressure. Toluol was added to all three, and then they were corked and incubated at room temperature for 25 days. After this time the liquids were decanted from the three tubes and saved, and the shavings were boiled in absolute alcohol for 15 minutes. When the alcohol was tested with phloroglucin acidified with HCl, a deep red color was given in the first tube, but the second and third remained clear and colorless. This is Czapek's (1) test for hadromal, and is an indication that *Trametes subrosea* liberates the extracellular enzyme, ligninase, which has the power of splitting lignin. Czapek says that lignin is a cellulose-hadromal ether which may be split by an enzyme.

The aqueous solutions which were decanted from the shavings were tested with Fehling's solution. The first reduced Fehling's solution, while the second and third yielded no trace of copper. The results show that these reducing substances are due to enzyme action, and that they must be aldoses. These sugars may be formed from various substances. There is probably some tannin and amygdalin in prune wood. Besides this there is the hydrolysis of the cellulose to reducing sugars, as demonstrated by the following experiment which was conducted simultaneously with the above.

CELLULASE EXPERIMENT

Very small bits of filter paper were placed in three test tubes. To the first was added 10 c. c. of the enzyme dispersion; to the second, 10 c. c. of the enzyme dispersion which had been autoclaved; and to the third, 10 c. c. of distilled water. Toluol was added to all three, and they were corked and incubated for 25 days.

After this the liquid was decanted and tested with Fehling's solution. The first tube gave a heavy precipitate of copper oxide, while there was not a trace in the second and third tubes. This indicated that cellulase was present as an active agent in the mycelium of *Trametes subrosea*.

HEMICELLULASE EXPERIMENT

Small chips of the paragalactan from the endosperm of date seeds were also used as a substrate in the same way as described above for prune-wood shavings and filter paper. In this case, when the first tube was tested with Fehling's solution a heavy precipitate of copper oxide was given, but none was evident in the control tubes. This demonstrates the presence of hemicellulase, an enzyme which splits the paragalactan, a hemicellulose, yielding a mixture of arabinose and galactose, both of which reduce Fehling's solution.

This combination of cytohydrolytic enzymes from the mycelium of *Trametes subrosea* is the explanation of the brown rot resulting from its wood-destroying activity in the peach and prune wood. Doubtless, also, the presence of emulsin makes available more food which exists in the wood in the form of glucosides. This ability of this fungus to make available as food these substances in rapidly grown orchard trees may be a partial explanation for the very rapid and devastating advancement of such forest parasites on these trees. For, it is a recognized fact that such forest-tree wound parasites as those represented by *Trametes subrosea* attack orchard trees more energetically than they seem to attack forest trees. Whether carbohydrates are more plentiful or more easily made available in the rapidly grown wood of orchard trees is not known.

DAMAGE TO ORCHARD TREES

The damage done by this heart rot is not easily estimated, because of the inability to estimate the cost of bring trees into bearing and the cost of replacing trees made decrepit through the influence of heart rot produced by *Trametes subrosea*. Other difficulties arising in such estimates of damage are the many different phases of the damage. In the first place the wood decay is not limited to the heart wood. Especially is this true of prune trees. After a tree has been affected for a number of years, the decayed cylinder of wood increases in diameter until the amount of sapwood is relatively small. In such cases it is not uncommon to find (especially if one side of a tree has been affected by winter injury) long sunken areas where the decayed wood extends to the bark. These may extend well up into smaller branches. This encroachment upon the sapwood materially arrests the desired increment of bearing wood on the branches affected, or on all of the branches above such a sunken area on the trunk.

Wood decay produced by *Trametes subrosea* has a marked influence on the neighboring sapwood. In the healthy sapwood of both peach and prune, wood tyloses are found scatteringly in the larger vessels of the spring wood only, none having been found in the smaller vessels of the summer growth. Where only 3 to 6 annual rings of sapwood separate the decayed cylinder within from the bark, tyloses were extremely numerous in the larger vessels of the spring wood in rings 2 or more years old. In fact, in prune wood especially, vessels of spring wood have been found nearly filled with tyloses, and tyloses were also numerous in the vessels of the summer wood. Where several annual rings separate the decayed heart and the last year's wood growth, the toxic influence of the decayed wood on the production of tyloses in vessels 2 or 3 years old is nearly as great as where

the fungus is nearer. Stevens (3) has described a similar condition in *Catalpa* sapwood influenced by the decay produced by *Polystictus versicolor* in closely neighboring tissue.

Such disturbances surely influence the normal physiology of a tree, bringing about a weakened framework, increasing the mortality of crop-producing wood, and causing increased susceptibility to winter injury. There are also indications that the lack of water conduction in wood having such an abundance of tyloses has a direct contributory bearing on the physiological disturbances in prune trees known as leaf roll, June drop of fruit, and early yellowing and dropping of leaves.

An orchard survey was made to ascertain just how much damage is done by the wood decay caused by *Trametes subrosea*. Out of 1,261 prune trees in orchards having average horticultural care, 1,212 showed heart rot. Of these, 885 were affected by *T. subrosea*. That is, 73 per cent of the heart rot found in the orchards is caused by *T. subrosea*. Of the 885 trees affected with this disease, 619 (or about 70 per cent) had pruning cuts 2 inches or more in diameter. These pruning cuts had not been treated with an antiseptic wound dressing.

In Douglas County, Oreg., 100 trees were examined in each of two neighboring orchards of Italian prunes. One orchard showed extremely good care, all pruning cuts of about 1.5 inches in diameter having been treated with some type of asphaltum wound dressing. The other orchard showed lack of care in the protection of wounds. In the orchard showing good care, 91 per cent of the trees showed no evidence of wood decay, although 82 per cent of them had large pruning cuts. On the other hand, of the 100 trees in the orchard receiving meager care only 7 per cent were sound and all had large pruning cuts. Of the 93 trees showing wood decay, 78, or 83.9 per cent, had the brown-pocket rot due to *Trametes subrosea*, while all of the 9 trees showing decayed wood in the other orchard were affected by this organism.

In some localities within this active range of the fungus, vigorously growing older orchards do not seem to be affected by the heart rot. On river bottom land, such as that in the Days Creek Valley, Douglas County, Oreg., and in Clarke County, Wash., prune trees grow to large size. The lack of heart rot in many orchards of some of these communities seems to be related to the system of pruning adopted by the grower. In brief, heart rots are less prevalent in orchards where the system of pruning used does away with all pruning on the main leader branches and trunk, particularly where trees are so trained in early years that the removal of large branches is unnecessary later.

PREVENTIVE MEASURES

To prevent the wood decay of stone-fruit trees caused by *Trametes subrosea*, the measures usually suggested for prevention of infection by wood-destroying fungi are recommended, i. e., pruning properly so that the wound will heal in the shortest possible time, and treatment with a wound dressing. The writer has had success with a Bordeaux paint made up by mixing linseed oil into a Bordeaux dust until a thick, smooth paint is formed. Preferably, however, it is better to educate growers to a pruning system whereby large

pruning cuts which need treatment are unnecessary or reduced to a minimum.

SUMMARY

Trametes subrosea Weir causes a brown-pocket heart rot of peach and prune trees in orchard districts from central California to British Columbia. The brown rot has been demonstrated to have been produced by the cytohydrolytic enzymes ligninase, cellulase, and hemicellulase. Emulsin is also present in the mycelium of *T. subrosea*, making available the products from glucoside digestion in the wood.

The economic losses due to this decay in orchard trees are large but difficult to estimate. Also, the damage to the diseased tree is not limited to the heartwood, since the disease encroaches upon the sapwood, cutting down its volume and actually destroying the water conducting power of the remaining sapwood by the stimulation of the growth of tyloses in the xylem vessels, upsetting the physiological balance in the tree, and giving rise to "drouth effects" in the affected portions of the tops.

A survey in prune orchards showed 73 per cent of the heart rot to be produced by *Trametes subrosea*, and demonstrated that the presence of this heart rot is related to large, unprotected pruning cuts.

Preventative measures are (1) proper pruning to facilitate healing, (2) treating the wounds with an antiseptic dressing, and preferably (3) the use of a system of pruning such that large pruning cuts are unnecessary or are reduced to a minimum.

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THE WATER-SOLUBLE CONTENT OF CALCIUM AND PHOSPHORUS IN CABBAGE¹

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INTRODUCTION

In the preservation of plant materials by natural fermentation processes, such as the making of silage and sauerkraut, the quantity of nutrients expressed from the plant tissue must to a large extent determine the rate at which the fermentation proceeds. It is not merely sugar but also soluble nitrogen and salts that are involved in the development of the acid-producing bacteria. Of the mineral elements in the expressed juice, it is probable that calcium and phosphorus are among the most important.

For this reason it is of interest to know what percentage of these mineral elements is soluble in water. The present study is concerned with the soluble calcium and phosphorus of cabbage, the ratio which each bears to the total quantity of the particular element, and the variation of each with the type of soil and the maturity of the plant. Incidentally, the data for 1925 will be compared with those reported in a previous paper (8)² showing the variations in the total calcium and phosphorus of 18 samples of cabbage grown during the season of 1924 in various parts of the United States.

EXPERIMENTAL PROCEDURE

Most of the cabbage was grown on two types of soil; an upland sandy loam which had been cropped for many years and an alkaline marsh soil which had been drained and kept under cultivation for about 10 years.

The cabbage was grown for kraut-making purposes, and from 50 to 60 heads were taken at each time of harvesting. The heads were stripped of the outer green leaves and cut into long shreds in a factory kraut cutter. From 100 to 125 pounds of shredded cabbage were obtained each time in this way. After mixing the whole quantity thoroughly to obtain uniformity, a sample of 400-gm. weight was moistened with ether, ground in a large mortar with sharp sand, and repeatedly extracted with fresh portions of water. The cloudy extract was filtered through paper pulp on a Büchner funnel and came through perfectly clear. After washing with several portions of water, the filtrate and washings were made up to 2,000 c. c. The above procedure is essentially that described by Tottingham, Schulz, and Lepkovsky (9) for extracting the nitrogenous constituents from plant cells. Three hundred c. c. aliquots were taken for calcium determinations and 100 c. c. for phosphorus.

Other portions of the cabbage were dried for the determination of moisture, calcium, and phosphorus. Moisture was ascertained by

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² Reference is made by number (italic) to "Literature cited," p. 699.

drying at 100° C. Both total and soluble calcium were determined by the McCrudden (3, 4) method as used when iron is contained in the sample. The two forms of phosphorus were obtained by the volumetric method of the Association of Official Agricultural Chemists (2, p. 3).

In Table 1 are given the chief facts regarding the variety, date of harvest, moisture and nitrogen content of the cabbage, and the type of soil on which the cabbage was grown. The samples are arranged so as to bring those from the same plot of ground together in one group, and the samples within the group are listed according to the date of harvest. The first sample was taken on September 16 when the cabbage was very green and not fully grown. Owing to unexpected freezing weather, the samples taken on November 11 were frozen solid and had been in this condition for four to five days before they were brought in from the field.

As was noted in a previous paper (6), the nitrogen content increased with maturity. This is particularly marked in the Miami silt-loam samples.

TABLE 1.—Description of cabbage samples

Sample No.	Variety of cabbage	Type and location of soil plot	Date of harvest	Moisture	Nitrogen
				<i>Per cent</i>	<i>Per cent</i>
349	All Season	Alkaline marsh, Madison, Wis.	Sept. 29, 1925	93.8	3.87
352	do.	do.	Oct. 23, 1925	93.6	3.33
355	All Season (frozen)	do.	Nov. 11, 1925	93.9	3.86
350	All Season	Miami silt loam, Madison, Wis.	Oct. 6, 1925	93.1	3.09
354	do.	do.	Oct. 28, 1925	92.9	3.41
356	All Season (frozen)	do.	Nov. 11, 1925	93.8	3.94
347	Glory	Clyde silt loam, Racine, Wis.	Sept. 16, 1925	93.2	1.92
348	All Head	do.	do.	93.3	2.00
351	All Season	do.	Oct. 15, 1925	93.2	2.95
353 ^a	do.	do.	Oct. 28, 1925	92.9	2.95
357	do.	Franksville, Wis.	Nov. 5, 1925	93.5	3.16
358	All Season (frozen)	do.	do.	93.8	3.38
Average				93.4	3.16

^a Same lot of cabbage as No. 351, stored 13 days.

VARIATIONS IN TOTAL AND WATER-SOLUBLE CALCIUM

In Table 2 are given the date for the calcium content of the samples. The percentage of calcium in the fresh vegetable ranged from 0.038 to 0.053 with an average of 0.046. In the paper previously reported one sample ran as low as 0.029, but the other 17 samples fell within the limits of 0.038 and 0.056 per cent and averaged 0.043 per cent. The results for the two years 1924 and 1925 are unexpectedly close to one another, especially when it is considered that the samples for the two years were grown on different types of soil and in different parts of the country. The marsh samples contained approximately one-third more calcium than the upland samples. This excess is probably related to the higher calcium content in the soil. Both of these sets of samples showed a drop in the calcium content of the second cutting as compared to the first; while the third cutting in both cases not only regained this difference, but slightly exceeded the first cutting in percentage of calcium. The Racine samples also showed a tendency toward higher calcium in the late samples than in the early ones. Less importance can be attached to these figures, however, since all the samples were not of the same variety of cabbage.

TABLE 2.—Total and water-soluble calcium of cabbage

Sample No.	Calcium in dry matter		Per-centage of total calcium extracted by water	Calcium in fresh vegetable	Sample No.	Calcium in dry matter		Per-centage of total calcium extracted by water	Calcium in fresh vegetable
	Total	Soluble				Total	Soluble		
	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>
349	0.81	0.49	60	0.050	348	0.57	0.37	65	0.038
352	.80	.46	57	.051	351	.63	.41	65	.043
355	.86	.36	42	.053	353	.65	.32	50	.046
350	.65	.44	67	.042	357	.70	.44	63	.046
354	.60	.35	58	.043	358	.73	.45	61	.045
356	.75	.46	61	.047					
347	.66	.45	68	.045	Average	.70	.42	60	.046

As the cabbage matures the percentage of water-soluble calcium decreases. All the samples show a change from soluble to insoluble forms of calcium as the season advances, but the marsh samples manifest this change to the most marked degree. This cabbage contained 60 per cent of the total calcium in a water-soluble form on September 29 and only 42 per cent on November 11. The effect of storage on water-soluble calcium is shown by samples 351 and 353. Storage for 13 days reduced the water-soluble calcium from 65 to 50 per cent of the total.

VARIATIONS IN THE TOTAL AND WATER-SOLUBLE PHOSPHORUS OF CABBAGE

In Table 3 are given the data for the phosphorus content of the samples. The maximum percentage in any sample of fresh vegetable is 0.036, the minimum is 0.023 and the average is 0.028. The variations in the 1924 samples were somewhat greater, extending from 0.017 to 0.037 per cent. This is not surprising since the 1924 experiment included a larger number of samples from more widely separated sources. The average for the earlier year, 0.024 is but slightly less than that for 1925. There appears to be a slight increase in total phosphorus as the crop matures, but the increase is too slight to warrant any definite conclusion.

TABLE 3.—Total and water-soluble phosphorus of cabbage

Sample No.	Phosphorus on dry basis		Per-centage of total phosphorus extracted by water	Phosphorus in fresh vegetable	Sample No.	Phosphorus on dry basis		Per-centage of total phosphorus extracted by water	Phosphorus in fresh vegetable
	Total	Soluble				Total	Soluble		
	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>
349	0.40	0.26	65	0.025	351	0.41	0.30	73	0.028
352	.38	.24	63	.024	353	.40	.20	50	.028
355	.44	.18	41	.027	357	.42	.27	64	.027
350	.43	.34	79	.030	358	.37	.28	76	.023
354	.50	.36	60	.036					
356	.53	.21	39	.033	Average	.43	.26	61	.028

As in the case of calcium, the soluble phosphorus decreases as the cabbage matures. In the Madison sets of samples the soluble phosphorus decreased from one-third to one-half of what it was when the earliest sample was taken. As shown by a comparison of samples 351 and 353, storage reduced the solubility of the phosphorus even more than it did that of calcium. Additional data are needed, however, to determine whether this is the general effect of storage.

The figures for soluble calcium and phosphorus are of the same order of magnitude as those which have been reported for plant materials by other investigators.

Ames and Boltz (1) found that 75 per cent of the phosphorus and 40 per cent of the calcium in alfalfa could be extracted by water. In a later paper Osborne, Wakeman, and Leavenworth (5) reported that 69.3 per cent of the inorganic matter of alfalfa was soluble in water, and that 49.2 per cent could be extracted by grinding and pressing the green plants. Of the latter 16.7 per cent was calcium and 5.5 per cent phosphorus.

In a study of the loss of mineral elements by various methods of cooking, Peterson and Hoppert (7) found that from summer cabbage 72 per cent of the calcium and 60 per cent of the phosphorus was extracted by boiling water.

In Table 4 are summarized the data for the two years 1924 and 1925. Although samples from the same year may vary from 50 to 100 per cent, the averages for the two years are very near together. In all but one case the averages for the two years differ by less than 10 per cent. The moisture figures for the two years are almost identical.

TABLE 4.—Two-year comparison of the average composition of cabbage, based upon 18 samples for 1924 and 12 for 1925

Constituent and range	1924	1925	Constituent and range	1924	1925
Calcium:			Total nitrogen:		
Maximum.....	<i>Per ct.</i> 0.056	<i>Per ct.</i> 0.053	Maximum.....	<i>Per ct.</i> 0.24	<i>Per ct.</i> 0.24
Minimum.....	.029	.038	Minimum.....	.15	.13
Average.....	.043	.046	Average.....	.19	.21
Phosphorus:			Moisture:		
Maximum.....	.037	.036	Maximum.....	93.9	93.9
Minimum.....	.017	.023	Minimum.....	91.0	92.0
Average.....	.024	.028	Average.....	92.6	93.4

SUMMARY

The percentage of calcium for 12 samples of cabbage ranged from 0.038 to 0.053 with an average of 0.046. On an average, 60 per cent of this calcium was soluble in water. The percentage of water-soluble calcium was highest in immature cabbage and decreased as the plant grew older.

The figures for phosphorus in these same samples varied from 0.023 to 0.036 per cent and averaged 0.028 per cent. The percentage of phosphorus soluble in water, 61, was almost exactly the same as that for water-soluble calcium. The solubility of the phosphorus decreased as the season advanced.

The average figures for these two elements for 1925 differ by less than 10 per cent from those obtained in 1924 from 18 samples of cabbage grown in some of the important cabbage-producing regions of the United States.

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PHOSPHORIC-ACID CONTENT OF CROPS GROWN UPON PEAT SOILS AS AN INDEX OF THE FERTILIZATION RECEIVED OR REQUIRED¹

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INTRODUCTION

Numerous European investigators, from the time of Hellriegel's work with barley in pot experiments in 1866 (10)³ to the present (16, 17), have tried to develop methods for the analysis of the soil by means of the plant—that is, from the content of plant nutrients in the dry matter of crops grown upon a soil to decide upon the adequacy of the supply of these nutrients in that soil for maximum crop production. Limiting values are obtained for selected crops by growing the plants under normal conditions of fertilization, gathering them at a certain stage of development, and analyzing the dry matter in order to ascertain the usual proportions of phosphoric acid, potash, and nitrogen. The same crops are also grown upon the soil under investigation, harvested at the same stage, and analyzed. Any marked deviation in the proportion of a nutrient in the dry matter, or in that of the ash, is taken as evidence of a deficiency or an abundance of this in an available form. Although these long continued efforts have so far failed to develop methods of general application, they have brought results that in many cases may prove to be valuable aids in studies of soil unproductivity.

Hall, from a detailed study of the composition of the ash of various crops grown on the Rothamsted plots (8), concludes that "by selecting suitable test plants valuable indications can be obtained as to the need or otherwise for specific manuring" (9, p. 177), root crops providing the most sensitive test plants—mangolds for potash and rutabagas for phosphoric acid. Cereals he regards as unsuitable as a rule, their ash constituents showing too narrow variations, the best among them as an indicator for both phosphoric acid and potash being the straw of barley.

Wagner, who probably has devoted more study to the subject than any other investigator, warns against overestimating the dependability of limiting values, even with the root crops and the

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³ Reference is made by number (italic) to "Literature cited," p. 739.

cereal straws, both of which show great differences in their ash content according to the fertilization they have received. He believes that a conclusion can be drawn as to the fertilizer requirements of the soil only where there are exceptionally high or exceptionally low percentages in the crop, and that by far the greatest number of cases do not belong to these extremes (26, p. 22-24).

It is in the case of meadows that Wagner has ventured on the most definite conclusions, and it is there that ash analysis gives promise of most usefulness. Where the phosphoric acid and potash needs are fully satisfied he finds that meadow plants give a hay that contains 0.65 per cent of phosphoric acid and 2 per cent of potash; the more the content sinks below these percentages the greater is the probability that heavier fertilization with phosphate and potash will increase the yields. By very great phosphoric-acid hunger he has found the content of this to fall as low as 0.2 per cent and with the heaviest phosphate fertilization to rise as high as 0.70 per cent (26, p. 155-156). If the potash content sinks as low as 1.20 per cent, it may be safely concluded that potash fertilization will certainly increase the yield, and a field experiment in such a case is unnecessary (26, p. 151). Wagner has dealt with the same subject in much detail in an earlier publication (25).

Schneidewind (20, p. 190-191) regards plant analysis as of value only in certain extreme cases, pointing out that at times an extraordinary variation, up to 300 per cent, is found in the percentage content of nutrients on the same field and in the same rotation, while by a change in the succession of crops even much greater variations can be obtained.

Most of the data reported in this paper were obtained incidental to the study of certain peculiarities and of some unexpected responses to fertilization of crops grown upon the peat soils of three Minnesota peat experimental fields—Golden Valley, Coon Creek, and Fens—all of which are naturally so abundantly supplied with lime that liming has been without beneficial effect even for those crops which are most sensitive to any deficiency, such as sweet clover. The experimental fields have been under operation since 1918, 1919, and 1915, respectively, and on all three the crops on the unfertilized peat have usually been so poor that in farming operations they would seldom have justified the expense of gathering, even in the first year of cultivation and when put in on a carefully prepared seed bed, but with the proper fertilization excellent yields have been obtained. Nitrogen applications have been unnecessary for ordinary farm crops, upon the yields of which they show little, if any, effect, although greatly stimulating the early growth while the soil is still cold. Stable manure produces satisfactory yields, but with most crops it fails to cause any distinct increase over those obtained with phosphate alone at Golden Valley or with a combination of phosphate and potash at Coon Creek and Fens.

In order to be able to judge to what extent the composition of the crops may indicate the fertilization required, the yields both with and without fertilization should be known, and for this reason the yields are reported for the crops whose composition is dealt with in this article.

CROPS AT GOLDEN VALLEY

The Golden Valley peat experimental fields (2, 3), located near the northwestern corner of Minnesota and 270 miles northwest of St. Paul, appear to have been the first reported anywhere in the world upon which an application of phosphate alone sufficed to bring about maximum crop yields. Potash fertilization caused no improvement, and the yields with the combination of potash and phosphate have been similar to those with phosphate alone. This result in the case of all the crops seeded in the first season at Golden Valley, which was very evident on June 20, 1918, when the senior author made his first visit to the fields after the planting of the crops about the middle of May, was so unexpected that had he not, only two months earlier, himself prepared and tagged all the sacks of fertilizers for the different plots, and later applied all of the phosphate and most of the potash, he would have suspected that by mistake the potash had been applied to Series IV and the phosphate to Series II instead of the reverse as intended (fig. 1). The very un-

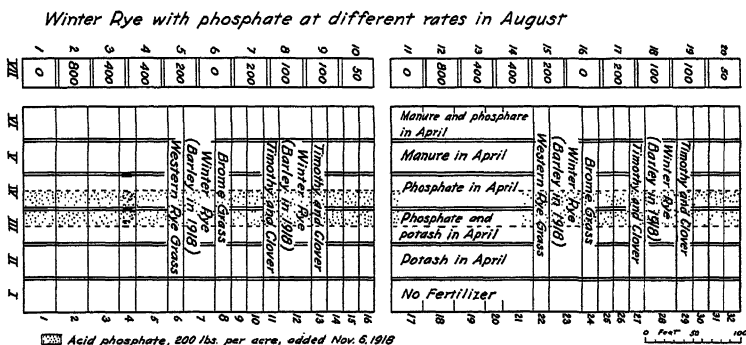


FIG. 1.—Diagram showing arrangement and fertilization of plots at Golden Valley sampled for analyses in 1919. All the fertilizers had been applied in 1918

pleasant caustic effect upon the hands and face of the potassium carbonate in the potash fertilizer employed had so heightened the vividness of the details of the application to each of the series in the recollection of a neighboring farmer who had applied the mixture to Series III, as well as to the senior author, that any such suspicion could not be entertained. A year later a disastrous flood, which destroyed all the crops except part of the winter rye and some of the hays, emphasized the desirability of a verification of the applications independent of personal recollections, and for this purpose the results of Wagner's work appeared to offer the most promise.

At the time the investigation was decided upon, late in the fall of 1919, the straw had been discarded from all the grain crops of the two seasons' work at Golden Valley. The first analyses were those of samples of hay which had been saved for moisture determinations, and the next of samples of small grains from the crop of 1918 which had been kept for exhibition purposes. These were followed by samples of rye from all the plots harvested in 1919.

HAY CROPS IN 1919

The arrangement and fertilization of the hay plots may be seen by reference to Figure 1. In April, 1918, the plots in Series IV had received 400 pounds per acre of 15 per cent acid phosphate and the plots in Series V had received 12 tons per acre of fresh stable manure. A month later all the hay plots were seeded without a nurse crop and a cutting taken at the end of August (Table 1). No more phosphate or manure had been applied before the samples reported in Table 1 were taken. The first of July, 1919, just as the hay was ready to be cut, the flood occurred and haying had to be delayed. After the water had subsided samples of the crop on four typical square yards on each plot were taken, cured in a well-aired barn loft, and later sent to the experiment station. On two sets of plots, Nos. 13 and 29, although alsike clover and redtop had been seeded along with the timothy,

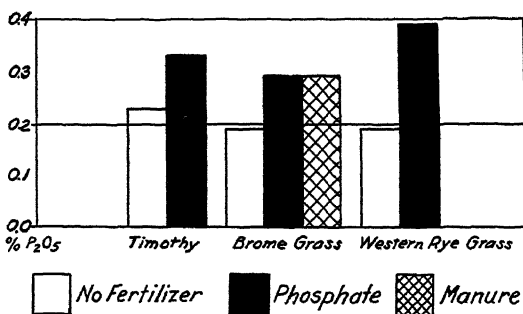


FIG. 2.—Effect of fertilization upon the phosphoric-acid content of dry matter of hays at Golden Valley in 1919

they formed an almost negligible proportion of the hay. The same was true of the red clover and redtop seeded on Nos. 11 and 27. So the crop from these four consisted almost exclusively of timothy.

TABLE 1.—Yield per acre of hay crops at Golden Valley

Hay crop	Yield in 1918			Yield in 1919		
	Unfer-tilized	With phos-phate	With manure	Unfer-tilized	With phos-phate	With manure
	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>
Brome grass.....	0.04	0.62	0.44	2.05	2.84	3.50
Western rye grass.....	.12	1.18	-----	2.11	3.69	-----
Timothy, with some red clover and redtop.....	.04	.84	-----	1.42	2.30	-----
Timothy, with some alsike and redtop.....	.04	.80	-----	1.57	2.17	-----

TABLE 2.—*Ash and phosphoric-acid content of hay crops grown at Golden Valley in 1919*

Crop and fertilization	Series	Plot	Ash in dry matter	P ₂ O ₅ in ash	P ₂ O ₅ in dry matter	Relative yield ^a
Timothy:			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
None.....	I	11	7.09	4.50	0.32	100
Do.....	I	13	5.77	3.29	.19	100
Do.....	I	27	6.24	3.69	.23	100
Do.....	I	29	6.02	2.99	.18	100
Average.....			6.28	3.62	.23	100
Phosphate.....	IV	11	4.45	8.09	.36	189
Do.....	IV	13	5.21	5.57	.29	150
Do.....	IV	27	5.17	6.38	.33	154
Do.....	IV	29	5.59	5.60	.33	124
Average.....			5.10	6.48	.33	154
Brome grass:						
None.....	I	8	6.04	3.64	.22	100
Do.....	I	24	4.97	3.22	.16	100
Average.....			5.50	3.43	.19	100
Phosphate.....	IV	8	5.06	5.13	.26	161
Do.....	IV	24	4.89	6.54	.32	130
Average.....			4.97	5.83	.29	145
Manure.....	V	8	5.23	5.35	.28	232
Do.....	V	24	4.96	6.25	.31	128
Average.....			5.09	5.80	.29	180
Western rye grass:						
None.....	I	6	3.99	5.51	.22	100
Do.....	I	22	4.17	3.84	.16	100
Average.....			4.08	4.67	.19	100
Phosphate.....	IV	6	3.47	12.96	.45	278
Do.....	IV	22	3.54	9.32	.33	121
Average.....			3.50	11.14	.39	199

^a The yield on the unfertilized plot of each pair is placed at 100.

The percentages of P₂O₅ and ash are reported in Table 2, and the effect of the various treatments upon the former is shown in Figure 2. The P₂O₅ content, whether referred to the dry matter or to the ash, was in all cases higher on the fertilized plots. In the crop from the unfertilized land it was so low as clearly to place this soil in the phosphate-deficient class, according to Wagner's conclusions mentioned above, but the hay from the phosphate-treated and from the manured land showed only from 0.26 to 0.45 per cent of P₂O₅ instead of the 0.65 per cent which Wagner considers the normal amount in hays from fields so liberally supplied with phosphate that larger applications will produce no further increase in yield. This would suggest that the initial application of phosphate should have been much heavier.

SMALL GRAINS IN 1918

The fertilization of the grain plots was the same as for the hay plots described above. At Golden Valley previous to the establishment of the experimental fields, the practice of burning had been developed locally by some of the peat-land owners (3, p. 98), and this was tried out along with other methods as a means of overcoming unproductivity. On the plots referred to as "burned," from 9 to 18 inches of peat had been burned off and on some the peat layer had been entirely destroyed. The resulting ash, with its abundance of available phosphate as well as much lime and a little potash, had been well mixed with the soil by plowing or disking. The burning was equivalent to an application of readily available phosphate many times as heavy as that applied in the form of acid phosphate to the phosphate-treated plots.

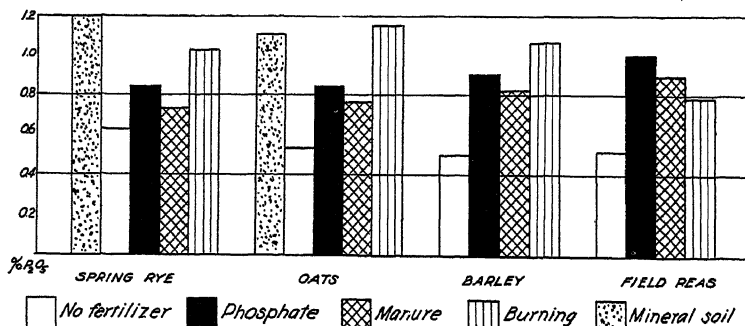


FIG. 3.—Effect of fertilization upon the phosphoric-acid content of small grains at Golden Valley in 1918

Each crop tried on the peat was also sown on the same day on an adjacent field of mineral soil, a black prairie loam, no application of manure or fertilizer being used.

The yields of grain are reported in Table 3 and the ash and P_2O_5 content in Table 4, and the latter is shown in Figure 3. Each sample analyzed was a composite from the duplicate plots, each 2 by 2 rods, and representative of the whole of each plot.

TABLE 3.—Yield per acre of small grains at Golden Valley in 1918

Crop	On peat soil				On unfertilized mineral soil
	No fertilizer	With phosphate	With manure	Burned	
	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>
Spring rye.....	14.6	39.6	36.8	59.7	23.9
Oats.....	32.8	81.3	72.1	66.4	39.5
Barley.....	7.4	24.6	25.3	42.4	27.5
Field peas.....	10.8	18.2	24.2	22.0	18.9

TABLE 4.—*Ash and phosphate content of small grains from Golden Valley in 1918*

Constituent and soil	Fertilization	Spring rye	Oats	Barley	Field peas
Ash in dry matter:		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Peat.....	None.....	1.87	2.96	2.79	2.71
Do.....	Phosphate.....	2.03	2.81	2.69	3.51
Do.....	Manure.....	1.84	3.01	3.01	3.39
Do.....	Burned.....	2.17	3.07	2.99	3.36
Mineral.....	None.....	2.43	3.40		
Phosphoric acid in ash:					
Peat.....	None.....	33.69	17.91	17.90	19.56
Do.....	Phosphate.....	41.33	29.89	33.83	28.50
Do.....	Manure.....	40.22	25.58	27.24	26.26
Do.....	Burned.....	47.60	37.46	35.45	23.21
Mineral.....	None.....	49.34	32.47		
Phosphoric acid in dry matter:					
Peat.....	None.....	.63	.53	.50	.52
Do.....	Phosphate.....	.84	.84	.91	1.00
Do.....	Manure.....	.74	.77	.82	.89
Do.....	Burned.....	1.02	1.15	1.06	.78
Mineral.....	None.....	1.20	1.10		
Relative yields of grain: ^a					
Peat.....	None.....	100	100	100	100
Do.....	Phosphate.....	271	247	442	169
Do.....	Manure.....	252	219	342	224
Do.....	Burned.....	312	162	576	179
Mineral.....	None.....	163	120	371	175
Relative yields of straw: ^a					
Peat.....	None.....	100	100	100	100
Do.....	Phosphate.....	233	304	267	227
Do.....	Manure.....	247	269	306	218
Do.....	Burned.....	248	338	486	153
Mineral.....	None.....	100	115	163	128

^a The yield on the unfertilized peat soil is placed at 100.

In the case of all four grains from the peat the P_2O_5 was much higher with the three treatments that supplied phosphate. In three of the four cases it was highest in the grain from the burned plots, but even on these it was only about like that in the grains from the mineral soil.

WINTER RYE IN 1919

In the season of 1919 winter rye was the only grain crop that matured, the others having been killed before maturity by the July flood (3, p. 51). Even the rye was injured, and showed a weight per bushel of only 50 to 52 pounds on the best fertilized plots, but on those receiving phosphate or manure it averaged, nevertheless, about 20 bushels per acre.

There were two distinct experiments with the rye, in one of which it was sown on plots that had produced barley in the crop season of 1918 (fig. 1, Series I to VI, plots 7, 12, 23 and 28), while in the other, involving the use of different quantities of acid phosphate, the rye was sown on land that had not previously been under experiment (fig. 1, Series VII, plots 1 to 20). In the case of both experiments, 4 square yards had been harvested from each plot, these samples threshed, and the grain saved.

On the plots that produced barley in 1918 the stubble had been plowed right after harvest, worked down, and seeded to rye without additional fertilization. Early in November a top dressing of 200 pounds per acre of 15 per cent acid phosphate was applied to the north half of the plots that five months before had received phosphate alone and to the south half of those that had been given potash as well as phosphate. Thus these two series of fortieth-acre plots had, in effect, been subdivided into four series of eightieth-acre plots. The application of potash in April, 1918, both alone and with phosphate, consisted

of 500 pounds per acre of Nebraska potash salts carrying 28 per cent potash, largely in the form of carbonate. The manure-with-phosphate application consisted of 12 tons per acre of manure with 400 pounds of the phosphate, and it also was added in April, 1918. In this experiment in the case of each treatment there are analyses of four samples, each from a different plot, except with the manure and phosphate, where there were samples from only two. The yields are reported in Table 5 and the content of ash and P_2O_5 in Table 6, while the last is shown graphically in Figure 4.

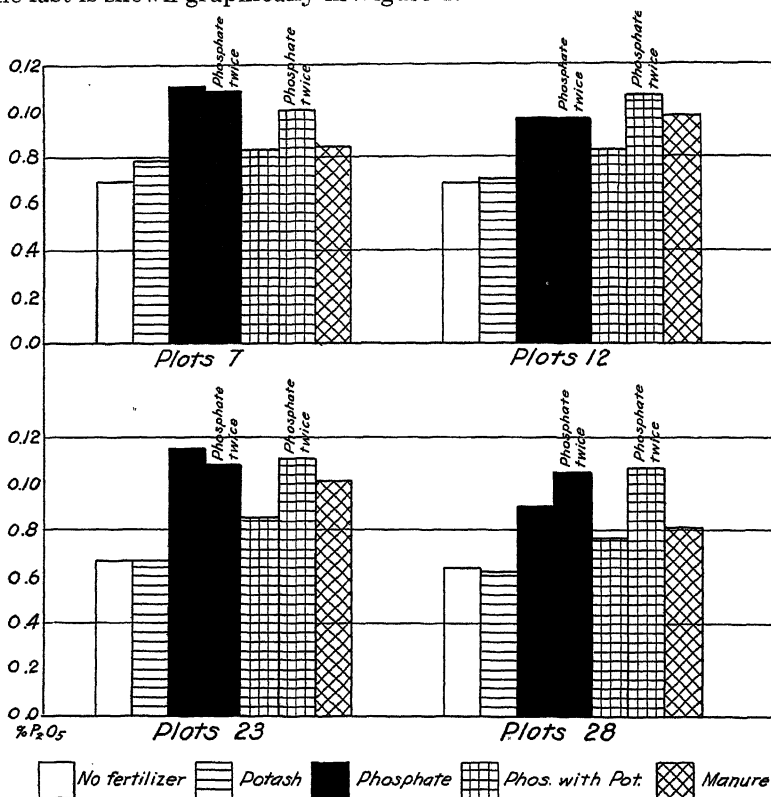


FIG. 4.—Effect of fertilization upon the phosphoric-acid content of winter rye at Golden Valley in 1919

TABLE 5.—Yields per acre of winter rye at Golden Valley in 1919 on plots that had produced barley in 1918

Plot No.	With no fertilizer	With potash	With phosphate applied once	With phosphate applied twice	With potash and phosphate once	With potash and phosphate twice	With manure	With manure and phosphate
	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>
7	3.3	3.7	28.8	27.5	19.1	25.4	23.1	
12	4.5	4.5	18.6	19.2	23.6	20.4	21.0	
23	3.5	2.8	17.6	20.4	21.8	18.3	20.3	17.8
28	3.7	3.8	11.6	13.0	16.4	10.2	12.7	18.0
Average	3.7	3.7	19.1	20.0	20.2	18.6	19.3	17.9

TABLE 6.—*Ash and phosphate content of winter-rye grown at Golden Valley in 1919 on peat soil that had produced barley in 1918*

Constituent and plot No.	With no fertilizer	With potash	With phosphate applied once	With phosphate applied twice	With potash and phosphate applied once	With potash and phosphate applied twice	With manure	With manure and phosphate
Ash in dry matter:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
7-----	2.53	2.48	2.25	2.18	1.98	1.78	1.85	-----
12-----	1.78	1.68	2.12	2.08	1.78	2.08	1.92	-----
23-----	1.81	1.88	2.35	2.26	1.95	2.22	2.15	2.25
28-----	1.78	1.65	2.02	2.25	1.85	2.28	1.98	2.05
Average-----	1.99	1.92	2.18	2.19	1.89	2.09	1.98	2.15
Phosphoric acid in ash:								
7-----	26.74	31.45	48.88	49.54	41.92	56.18	45.40	-----
12-----	38.20	41.66	45.28	46.15	46.01	50.96	50.51	-----
23-----	37.02	35.64	48.93	47.79	44.10	50.00	46.97	47.10
28-----	35.95	37.58	44.55	46.66	41.62	46.93	40.91	45.85
Average-----	34.48	36.58	46.91	47.53	43.43	51.02	45.95	46.47
Phosphoric acid in dry matter:								
7-----	.69	.78	1.10	1.08	.83	1.00	.84	-----
12-----	.68	.70	.96	.96	.82	1.06	.97	-----
23-----	.67	.67	1.15	1.08	.86	1.11	1.01	1.06
28-----	.64	.62	.90	1.05	.77	1.07	.81	.94
Average-----	.67	.69	1.03	1.04	.82	1.06	.91	1.00
Relative yields of grain: ^a								
7-----	89	100	778	743	516	686	624	-----
12-----	122	95	503	519	638	551	568	-----
23-----	95	76	476	551	569	495	549	481
28-----	100	103	314	351	441	276	343	486
Average-----	100	93	518	541	546	502	521	483
Relative yields of straw: ^a								
7-----	105	100	306	384	322	349	279	-----
12-----	125	106	253	312	297	292	271	-----
23-----	97	72	254	260	238	215	266	275
28-----	73	74	156	184	174	120	174	221
Average-----	100	88	242	285	258	244	248	248

^a The average yield on the four unfertilized plots, 3.7 bushels of grain and 924 pounds of straw per acre is placed at 100.

The P_2O_5 content was not affected by the potash application, but was much increased by both phosphate and manure. The second application of phosphate in some cases caused a definite increase in the P_2O_5 content compared with the single application, but in other cases no effect was noticed. In the grain from the plots that received potash along with phosphate in April, 1918, but no phosphate afterwards, there was less P_2O_5 than with any of the other fertilizers supplying phosphate.

In the other experiment, just prior to sowing the rye on land not previously fertilized, acid phosphate was applied at rates of 50 to 800 pounds per acre. The 20 plots were each 2 by 2 rods and formed a long series, shown as VII in Figure 1. The yields on the unfertilized plots were somewhat better than those on Series I in the experiment just described, which may be attributed to the fact that there was no removal of P_2O_5 from VII by cropping in 1918. The heavier applications caused the highest yields (Table 7).

TABLE 7.—*Ash and phosphoric-acid content of winter rye grown in 1919 at Golden Valley on peat soil fertilized with acid phosphate applied at different rates*

Constituent and order of plots	Rate per acre of acid-phosphate application					
	None	50 pounds	100 pounds	200 pounds	400 pounds	800 pounds
Plot numbers:						
1.....	1	10	8	5	3	2
2.....	6	20	9	7	4	12
3.....	11		18	15	13	
4.....	16		19	17	14	
Ash in dry matter:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	1.81	1.81	1.87	2.45	2.73	2.34
2.....	2.26	1.78	2.18	2.18	2.35	2.41
3.....	2.16		1.95	2.15	2.32	
4.....	1.71		2.38	2.15	2.45	
Average.....	1.98	1.79	2.09	2.23	2.46	2.38
Phosphoric acid in ash:						
1.....	35.36	35.91	37.44	37.15	38.83	45.73
2.....	31.86	37.64	39.00	40.36	39.57	45.64
3.....	33.34		38.46	42.32	45.69	
4.....	40.94		32.34	40.93	42.04	
Average.....	35.37	36.77	36.81	40.19	41.53	45.68
Phosphoric acid in dry matter:						
1.....	.64	.65	.70	.91	1.06	1.07
2.....	.72	.67	.85	.88	.93	1.10
3.....	.72		.75	.91	1.06	
4.....	.70		.77	.88	1.03	
Average.....	.69	.66	.77	.90	1.02	1.09
Relative yield of grain: ^a						
1.....	128	222	366	345	381	532
2.....	60	155	259	426	345	368
3.....	97		302	221	329	
4.....	115		182	378	255	
Average.....	100	188	277	342	327	450

^a The average yield on the 4 unfertilized plots, 5.8 bushels, is placed at 100.

The proportion of P_2O_5 was not affected by the 50-pound application and only slightly raised by the 100-pound fertilization, although with the latter the yield was nearly trebled. The heavier treatments with phosphate, however, distinctly raised the proportion in the grain, which reached as high values as on any of the older plots, Series I to VI, in either 1918 or 1919.

The failure of the lightest application to increase the P_2O_5 content is to be attributed to the extreme phosphate hunger of the rye, even after receiving this application. The yield of both grain and straw was at least doubled by the light phosphate application and about twice as much P_2O_5 was removed by the crop from an equal area, even though the percentage in the grain was no higher.

CROPS AT COON CREEK

The Coon Creek Peat Experimental Fields are only 20 miles northwest of St. Paul and of the three are the most accessible to the experiment station. Immediately adjacent to them are the Coon Creek Sand Experimental Fields. The peat is very unproductive unless fertilized with both potash and phosphate. Potash applied alone is slightly beneficial, but with applications of phosphate without accompanying potash the yields either remain unaffected or are somewhat depressed, indicating that the natural supply of P_2O_5 is better than that of K_2O . The crops of the seventh season were removed in 1925, and no distinct change in behavior has been observed. On two

adjacent series the same crops have been grown in a rotation every year, the one series receiving both phosphate and potash each spring and the other remaining unfertilized. The first has continued highly productive, whereas the other continues unproductive. The unfertilized series shows no distinct decline year by year; in the seventh season the crops are as good as in the first—as though the decay of the peat each season sets free enough P_2O_5 and K_2O to produce a very light crop.

The adjacent sand fields, although decidedly lime deficient, show little response to potash and still less to phosphate fertilization.

Except where otherwise indicated the crops analyzed were on land that had been tile-drained in October, 1918, broken during the following month, and worked down in April, 1919.

HAY CROPS IN 1920

In May, 1919, a mixture of timothy, red clover, and alsike was seeded without a nurse crop on four plots—one unfertilized, one given phosphate only, another potash only, and the fourth the combination of phosphate and potash. The phosphate applications consisted of 400 pounds per acre of 16 per cent acid phosphate in May, 1919, and half as much in April, 1920. The potash applications were 500 pounds per acre of 28 per cent Nebraska potash salts in May, 1918, and 100 pounds per acre of muriate of potash in April, 1920. A cutting of hay was taken at the end of August, 1919. On all the plots the stand of both the clovers and the timothy was excellent, and during the winter suffered no injury. On June 19, 1920, when the plants were about half grown, 50 of timothy and 50 of each of the clovers were cut off even with the ground for analysis. Although the combination of potash and phosphate more than doubled the yield in both seasons, neither caused any increase when used alone. The P_2O_5 content (Table 8) was raised by the use of phosphate alone and unaffected by the potash alone or by the combination of potash and phosphate. Although the combination failed to distinctly increase the percentage of P_2O_5 , it trebled the yield of hay, and hence trebled the amount of P_2O_5 removed in the crop from a unit area.

TABLE 8.—*Ash and phosphoric acid in plants from clover-timothy meadow on peat soil at Coon Creek in 1920*

Constituent and plants	With no fertilizer	With potash	With phos- phate	With potash and phos- phate
Ash in dry matter:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Red clover.....	6.70	6.68	6.73	7.75
Alsike clover.....	7.58	7.60	7.58	7.38
Timothy.....	3.75	4.13	3.48	3.55
Phosphoric acid in ash:				
Red clover.....	5.37	5.39	8.17	5.16
Alsike clover.....	5.93	5.53	7.52	5.96
Timothy.....	6.93	7.26	10.64	-----
Phosphoric acid in dry matter:				
Red clover.....	.36	.36	.55	.40
Alsike clover.....	.45	.42	.57	.44
Timothy.....	.26	.30	.37	-----
Yields per acre of hay:	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>
Crop of 1919, Sept. 25.....	1.07	.99	.77	2.42
First crop of 1920, July 3.....	1.16	.41	.84	2.47
Second crop of 1920, Aug. 30.....	.87	.66	.90	2.24
Sum of 2 crops in 1920.....	2.03	1.07	1.64	4.71

Two plots near those just dealt with were given a coating of sand, at the rate of 300 tons per acre, early in July, 1918, and a few days later, along with many other plots, seeded with a mixture of timothy, alsike, and red clover, a good stand resulting. Late in September of the same year the plots were mowed, the yield of dry hay being 0.74 ton per acre on the one and 0.64 on the other, compared with 0.28 and 0.37 ton on the corresponding plots receiving neither fertilizer nor sand. Early in the following May each plot was subdivided into four parts, one of which was left unfertilized, one treated with potash only, another with phosphate only, and the third with the combination of potash and phosphate. In 1920 two cuttings of hay were taken, and the sanded plots, even those unfertilized, yielded nearly four times as much as the corresponding plots that had received neither sand nor fertilizer. Potash increased the yield on the sanded plots nearly as much when applied alone as when combined with phosphate. On June 19, when the plants were about half grown, samples composed of 50 typical plants of the timothy and the two clovers were taken.

TABLE 9.—*Ash and phosphoric acid in plants from clover-timothy meadow on sanded peat soil at Coon Creek in 1920*

Constituents and plants	With no fertilizer	With potash	With phos- phate	With potash and phos- phate
Ash in dry matter:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Red clover.....	7.15	7.33	6.05	6.83
Alsike.....	5.78	7.28	6.25	7.30
Timothy.....	3.90	4.38	3.88	4.95
Phosphoric acid in ash:				
Red clover.....	3.07	4.23	6.60	5.85
Alsike.....	8.99	6.18	10.56	8.36
Timothy.....	8.97	7.08	12.11	9.29
Phosphoric acid in dry matter:				
Red clover.....	.22	.31	.40	.40
Alsike.....	.52	.45	.66	.61
Timothy.....	.35	.31	.47	.45
Yield per acre of hay in 1920:	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>	<i>Tons</i>
First cutting.....	2.29	3.11	1.82	3.92
Second cutting.....	1.20	1.51	1.71	1.98
Sum of two cuttings.....	3.49	4.62	3.53	5.90

The P_2O_5 content (Table 9) of the dry matter bears much the same relation to the fertilization as on the unsanded meadow, except that here the combination of potash and phosphate raised the percentage practically as much as the phosphate alone.

RYE HAY IN 1920

Winter rye plants were gathered on June 11, 1920, from plots on the peat and on the adjacent sand experimental field. On each four treatments are represented, namely, none, potash, phosphate, and potash with phosphate. On the peat the fertilizer applications were the same as on the timothy-clover plots above, and on the sand they were about the same. On the peat potatoes had been grown in 1918, the rye having been sown as soon as these were dug, that is, early in

September; on the sand the rye had been preceded by a clover meadow.

The average weight of the culms reported in Tables 10 and 11 does not indicate the relative production of dry matter on the different plots, as the culms were much more numerous as well as heavier on the plots receiving the proper fertilization. The yields of the mature crop, reported in the last section of the tables, very well indicate the relative growth on the different plots at the time of sampling.

TABLE 10.—*Ash and phosphoric-acid content of winter rye hay from peat soil at Coon Creek in 1920*

Plot	With no fertilizer	With potash	With phos- phate	With potash and phos- phate
Ash in dry matter:				
East.....per cent.	5.04	6.08	5.70	4.90
West.....do.	4.82	6.20	4.45	4.84
Average.....do.	4.98	6.14	5.07	4.87
Phosphoric acid in ash:				
East.....do.	8.03	4.44	14.04	11.43
West.....do.	9.34	5.00	12.81	8.88
Average.....do.	8.68	4.72	13.42	10.15
Phosphoric acid in dry matter:				
East.....do.	.45	.27	.80	.56
West.....do.	.45	.31	.57	.43
Average.....do.	.45	.29	.68	.49
Dry weight of 100 culms:				
East.....grams.	19	25	15	39
West.....do.	26	40	38	50
Average.....do.	22	32	26	44
Ratio of green weight to dry weight:				
East.....do.	4.3	4.1	4.3	3.6
West.....do.	4.0	4.2	3.9	4.3
Average.....do.	4.1	4.1	4.1	3.9
Relative yields of dry matter in mature crop: ^a				
East.....per cent.	66	184	64	287
West.....do.	133	265	185	399
Average.....do.	100	224	124	343

^a The average of the yields on the unfertilized plots, 1,450 pounds per acre, is placed at 100.

TABLE 11.—*Ash and phosphoric-acid content of winter rye hay from Coon Creek sand experimental field in 1920*

Plot	With no fertilizer	With potash	With phos- phate	With potash and phos- phate
Ash in dry matter:				
South.....per cent.	4.30	4.58	4.64	4.34
North.....do.	4.10	4.28	4.34	4.34
Average.....do.	4.20	4.43	4.49	4.34
Phosphoric acid in ash:				
South.....do.	13.26	12.23	12.93	13.36
North.....do.	12.44	14.25	13.13	13.59
Average.....do.	12.85	13.24	13.03	13.47

TABLE 11.—Ash and phosphoric-acid content of winter rye hay from Coon Creek sand experimental field in 1920—Continued

Plot	With no fertilizer	With potash	With phospho-phate	With potash and phospho-phate
Phosphoric acid in dry matter:				
South.....per cent..	0.57	0.56	0.60	0.58
North.....do.....	.51	.61	.57	.59
Average.....do.....	.54	.58	.58	.58
Dry weight of 100 culms:				
South.....grams..	41	37	40	46
North.....do.....	47	39	54	54
Average.....do.....	44	38	47	50
Ratio of green weight to dry weight:				
South.....	2.6	2.7	2.7	2.6
North.....	2.7	2.7	2.9	2.9
Average.....	2.6	2.7	2.8	2.7
Relative yields of dry matter in mature crop: *				
South.....per cent..	91	100	86	94
North.....do.....	108	93	111	96
Average.....do.....	100	96	98	95

* The average of the yields on the unfertilized plots, 2,823 pounds per acre, is placed at 100.

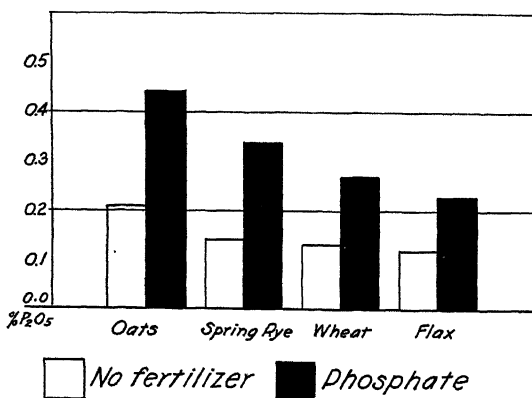


FIG. 5.—Effect of phosphate fertilization upon the phosphoric-acid content of dry matter of straw of small grains at Coon Creek in 1919.

From Tables 10 and 11 it will be seen that very different results were secured on the two fields. On the sand, on which none of the fertilization improved the growth, there was no distinct effect on the P_2O_5 content, while on the peat, on which the combination of phosphate and potash had greatly increased the growth, there were some marked effects, the phosphate alone distinctly raising the P_2O_5 , the potash alone as distinctly lowering it, and the combination giving intermediate values so similar to those obtained on the unfertilized plots that the treatment of the plots could not have been identified from the analyses. On the sand the P_2O_5 content was comparatively high on all the plots and the phosphate fertilization did not distinctly increase it.

It is of interest to note that the P_2O_5 content of the rye hay from the unfertilized plots on peat at Coon Creek, 0.45 per cent, is the same as that on a phosphate-treated plot at Golden Valley (Table 22), from which samples were collected on the following day, and much higher than that of the unfertilized rye at the latter place.

STRAW OF SMALL GRAINS IN 1919

In Table 12 are reported the yields and the P_2O_5 content of the straw of oats, spring rye, wheat, and flax grown at Coon Creek in 1919, both without any fertilizer and with phosphate only. The fertilizers were applied and the seed sown early in May. The phosphate plots received 400 pounds per acre of 16 per cent acid phosphate. Both without any fertilizer and with phosphate only the yields were very poor. With each of the four crops the P_2O_5 content of the straw was about doubled by the fertilization (fig. 5).

Before this study was decided upon the straw from the two other treatments—potash only and potash with phosphate—had been discarded, but it happened that the sample sheaves of buckwheat from 15 plots of an adjacent experiment on the peat had not yet been threshed, and the samples of straw from all of these were analyzed, K_2O as well as P_2O_5 being determined.

TABLE 12.—*Phosphoric-acid content of straw of small grains grown on peat at Coon Creek in 1919*

Ash and P_2O_5 determinations, and yields	Oats		Spring rye		Wheat		Flax	
	Unfertilized	Fertilized with phosphate	Unfertilized	Fertilized with phosphate	Unfertilized	Fertilized with phosphate	Unfertilized	Fertilized with phosphate
Ash in dry matter, per cent.....	6.420	7.920	4.120	5.220	5.550	8.070	3.630	4.820
P_2O_5 in ash, per cent.....	3.350	5.570	3.520	6.480	2.340	3.410	3.310	4.730
P_2O_5 in dry matter, per cent.....	.215	.440	.145	.338	.130	.275	.120	.228
Yield of straw, in pounds per acre.....	1,253	1,707	321	349	153	145	2,239	3,496
Yield of grain, in bushels per acre.....	14.0	15.2	1.7	1.3	0.5	0.6	8.3	10.4

The land used in this experiment had been drained at the same time as that devoted to the preceding experiments, but only part of it had been broken before winter set in. The rest was plowed at the end of the following May and the whole used for the experiment with buckwheat, shown in Figure 6. The individual plots were very small, only 16 by 28 feet, necessitating great care in applying the fertilizers and in gathering the samples. Each sample was taken from 4 typical square yards, and the yields were computed from these samples. The seed was sown on three successive dates, June 13, June 23, and July 5, but all the fertilizers were applied and worked into the soil at the same time—that is, just before the first seeding.

The potash was applied in the form of 28 per cent Nebraska potash salts, 500 pounds per acre. The 16 per cent acid phosphate was used at the rate of 400 pounds per acre, and the treble superphosphate, with 48 per cent water-soluble phosphoric acid, at the rate of 133 pounds per acre.

The loss of grain from the first and second seedings through shattering in the field makes the data on yield of grain valueless. With the third cutting there was no serious shattering, and the yields are reported in Table 13. Neither phosphate alone nor potash alone benefited the crop, but a combination of the two doubled the yield of grain and greatly increased that of straw.

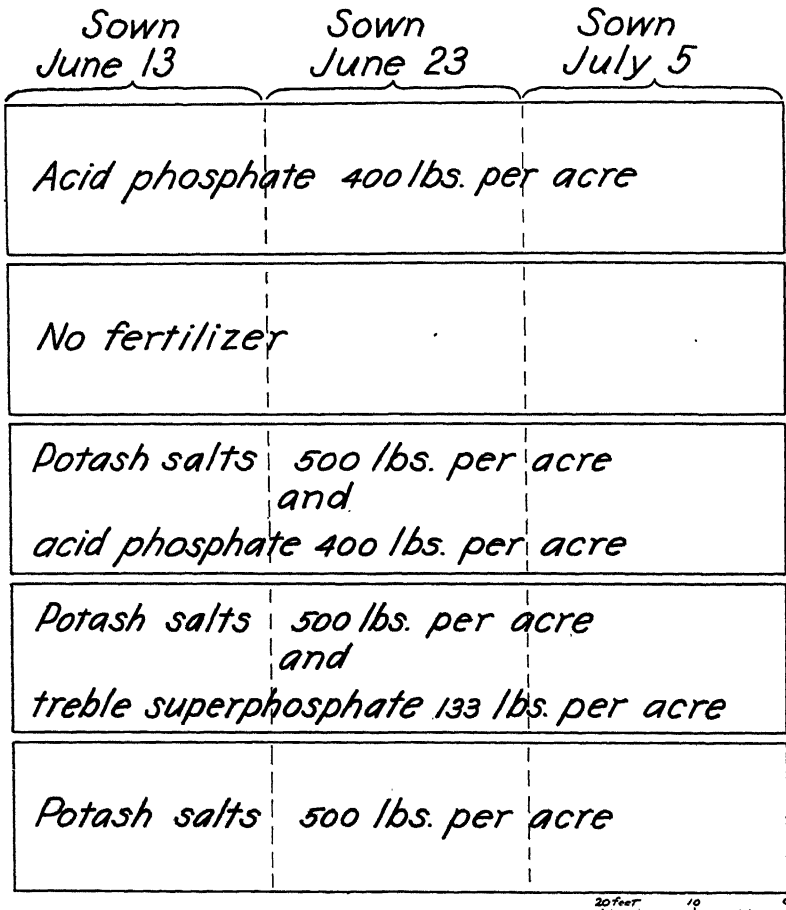


FIG. 6.—Diagram showing arrangement and fertilization of plots used in experiment with buck-wheat at Coon Creek in 1919

The P_2O_5 content of the straw was increased from three to ten fold by the application of phosphate, both alone and in combination with potash. The K_2O was doubled or trebled by the application of the potash salts, either alone or in combination (fig. 7).

TABLE 13.—Yields per acre from third seeding of buckwheat on peat at Coon Creek in 1919

Fertilization	Grain	Straw
	<i>Bushels</i>	<i>Pounds</i>
None.....	13.94	1,944
Acid phosphate.....	8.78	1,352
Potash salts.....	8.43	1,234
Potash salts with acid phosphate.....	27.02	3,091
Potash salts with treble superphosphate.....	26.81	3,291

STRAW OF SMALL GRAINS IN 1920

In the spring of 1920 a block of eight plots that had been fertilized the year before and planted to potatoes was refertilized and sown to small grains. On three of the plots the crops made a good growth,

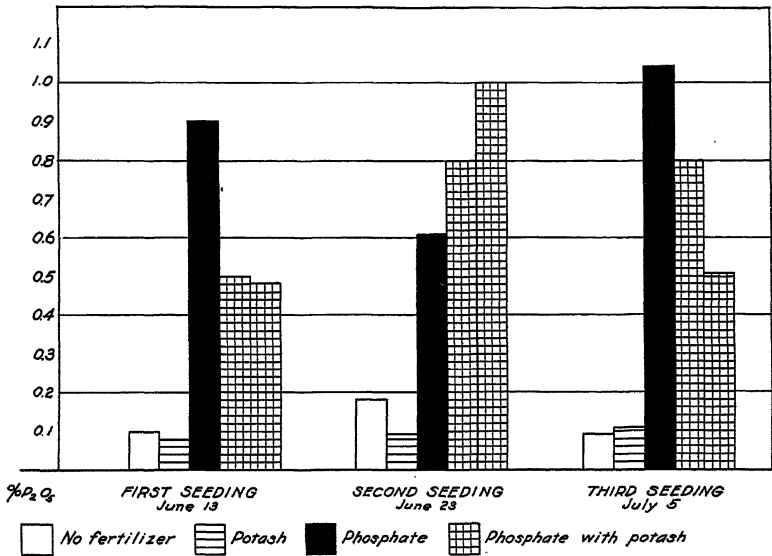


FIG. 7.—Effect of fertilization upon the phosphoric-acid content of dry matter of buckwheat straw at Coon Creek in 1919

until very heavy rains during the first part of July raised the water table almost to the surface, and it remained so high for several weeks, in the case of many of the plots, that the later growth was very poor. The injury was least on the most westerly plot, No. 1, which was nearest to a tile line, and increased up to No. 8, which was farthest from the drain. This accounts for the difference between the yields from duplicate plots and to some extent for the difference in the P₂O₅ content of the straw from duplicate plots.

Plot No. 1, which should have received only potash, showed such a good growth of various crops that it was suspected phosphate might have been applied to this by mistake. To get some information on this point, P₂O₅ determinations were made on such of the straws as could be identified, the tags from some having become detached.

TABLE 14.—*Composition of straw of buckwheat grown on peat at Coon Creek in 1919*

Constituent and seeding	With no fertilizer	With potash	With phos- phate	With potash and acid phos- phate	With potash and treble super- phos- phate
Ash in dry matter:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
First.....	5.17	6.45	4.78	5.64	5.58
Second.....	4.70	6.13	4.23	7.75	6.95
Third.....	5.30	5.51	4.60	8.15	6.58
Average.....	5.06	6.03	4.53	7.18	6.37
Phosphoric acid in ash:					
First.....	1.93	1.24	18.83	8.87	8.60
Second.....	3.83	1.47	14.47	10.32	14.38
Third.....	1.70	2.00	22.62	9.81	7.77
Average.....	2.49	1.57	18.64	9.67	10.25
Phosphoric acid in dry matter:					
First.....	.10	.08	.90	.50	.48
Second.....	.18	.09	.61	.80	1.00
Third.....	.09	.11	1.04	.80	.51
Average.....	.12	.09	.85	.70	.66
Potash in ash:					
First.....	28.43	36.60	22.60	47.87	49.62
Second.....	27.67	53.50	30.74	54.45	53.23
Third.....	27.93	64.20	19.57	53.00	49.54
Average.....	28.01	51.44	24.30	51.77	50.80
Potash in dry matter:					
First.....	1.47	2.36	1.06	2.70	2.77
Second.....	1.30	3.28	1.30	4.22	3.70
Third.....	1.48	3.54	.90	4.32	3.26
Average.....	1.42	3.06	1.09	3.75	3.24
Relative yields of straw: ^a					
First.....	100	129	92	145	137
Second.....	100	117	99	144	160
Third.....	100	63	69	169	160
Average.....	100	103	87	133	152

^a The yields on the unfertilized plots on the three dates, respectively, 3,159, 2,669, and 1,944 pounds per acre, are placed at 100.

TABLE 15.—*Phosphoric-acid content of grain straws grown on peat at Coon Creek in 1920*

Constituent and fertilization	Plot	Barley	Early oats	Late oats	Flax	Winter rye
Ash in dry matter:						
None.....	4	<i>Per cent</i> 4.90	<i>Per cent</i> 5.35	<i>Per cent</i> 5.51	<i>Per cent</i> 4.15	<i>Per cent</i> 3.56
Do.....	8	4.11	4.78	5.34	4.65	-----
Average.....		4.50	5.06	5.42	4.40	-----
Potash.....	1	5.47	6.75	6.19	2.82	8.92
Do.....	5	4.53	5.48	5.57	2.25	3.86
Average.....		5.00	6.11	5.88	2.53	-----
Phosphate.....	3	5.74	5.65	-----	2.80	4.63
Do.....	7	7.36	7.13	-----	6.90	5.76
Average.....		6.55	6.39	-----	-----	5.19
Phosphate and potash.....	2	4.94	6.06	5.43	-----	3.37
Do.....	6	6.70	5.15	5.84	4.16	6.70
Average.....		-----	5.60	5.63	-----	-----
Phosphoric acid in dry matter:						
None.....	4	.20	.09	.10	.06	.08
Do.....	8	.24	.11	.08	.07	-----
Average.....		.22	.10	.09	.06	-----
Potash.....	1	.10	.05	.07	.05	.10
Do.....	5	.14	.09	.05	.06	.09
Average.....		.12	.07	.06	.05	.09
Phosphate.....	3	.68	.24	-----	.31	.37
Do.....	7	1.29	.58	-----	1.25	.69
Average.....		.98	.41	-----	-----	.53
Phosphate and potash.....	2	.13	.32	.33	-----	.19
Do.....	6	.37	.21	.30	.31	.37
Average.....		-----	.26	.31	-----	.28
Yields per acre of straw and grain:		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
None.....	4	-----	2,170	2,540	1,350	1,930
Do.....	8	-----	730	1,330	670	970
Average.....		-----	1,450	1,935	1,010	1,450
Potash.....	1	-----	3,770	6,700	3,820	3,940
Do.....	5	1,480	2,570	4,120	2,450	2,680
Average.....		-----	3,170	5,410	3,135	3,310
Phosphate.....	3	-----	2,420	1,940	3,890	2,740
Do.....	7	970	1,450	1,810	1,290	920
Average.....		-----	1,935	1,875	2,590	1,830
Phosphate and potash.....	2	4,030	5,400	7,250	4,290	5,740
Do.....	6	-----	4,100	5,940	2,970	4,280
Average.....		-----	4,750	6,595	3,630	5,010

The effect of the applications (Table 15) was as marked as in the case of the straws in the preceding season (Tables 12 and 14). The very great accumulation of P_2O_5 in the straw on plots 3 and 7 was due to the very light growth of the crops on those plots. The very low content of P_2O_5 in the five samples from plot 1 makes it evident that no application of phosphate had been made to this by mistake, and if it received any phosphate the quantity must have been very small,

such as might be accounted for by the blowing upon it of ashes from a few rods just to the south, where in the spring the peat had taken fire during a strong south wind and burned for several hours.

TABLE 16.—*Phosphoric-acid content of buckwheat grown on peat at Coon Creek in 1919*

Constituent and seeding	With no fertilizer	With acid phosphate	With acid phosphate and potash
Ash in dry matter:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
First.....	1.71	2.07	2.05
Second.....	1.61	2.04	1.98
Third.....	1.59	2.07	1.95
Average.....	1.64	2.06	1.99
Phosphoric acid in ash:			
First.....	35.1	40.6	42.0
Second.....	36.6	41.2	42.9
Third.....	33.9	40.6	42.6
Average.....	35.2	40.8	42.5
Phosphoric acid in dry matter:			
First.....	.60	.84	.86
Second.....	.59	.84	.85
Third.....	.54	.84	.83
Average.....	.58	.84	.85

BUCKWHEAT IN 1919

The P_2O_5 and ash were determined in the grain from the buckwheat on nine of the plots described above (Table 16). There was an increase of about 40 per cent in the P_2O_5 content and of about 25 per cent in that of the ash, both with the combination of potash and phosphate and with the phosphate alone, although the yield with the former treatment was three times as heavy (Table 13). Here, as with the buckwheat straw (Table 14), the addition of the potash to the phosphate greatly increased the yield without lowering the percentage of P_2O_5 in the crop.

WINTER RYE AND FLAX IN 1920

The grain from the duplicate plots of winter rye in the experiment dealt with above (Table 15) was combined and analyzed for ash and P_2O_5 (Table 17). The percentages of both were somewhat depressed by potash alone and greatly increased by phosphate alone, as well as by the combination of phosphate and potash.

TABLE 17.—*Phosphoric-acid content of winter rye and flax grown on peat at Coon Creek in 1920*

Crop	Fertilization	Ash	P_2O_5 in ash	P_2O_5 in dry matter
Winter rye.....		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
	None.....	1.54	42.7	0.67
	Potash.....	1.42	40.9	.58
	Phosphate.....	1.90	51.6	.98
	Phosphate and potash.....	1.83	50.8	.93
Flax.....	None.....	3.56	42.1	1.50
	Phosphate and potash.....	4.54	43.2	1.96

Samples of flax grain from two plots were analyzed. Both had been cropped in 1919. One had received no fertilizer, while the other had been given the mixture of phosphate and potash in May, 1919, and again in April, 1920, as described above in connection with Table 9. The content of both ash and P_2O_5 was increased by the fertilization, the latter by 30 per cent.

In the same season flax was grown on 12 plots on a field of peat on the Radisson farm, 3 miles from the Coon Creek experimental fields. The peat on this field is very similar to that at Coon Creek and had been broken the preceding fall. The crops on this were not injured by the heavy rains during the early part of July.

The P_2O_5 content of the grain bears only a rather uncertain relation to the fertilization (Table 18). On the two unfertilized plots it was low, on that given only potash still lower, and highest on the plots receiving 400 or 800 pounds of phosphate without the maximum application of the potash. The yields of potatoes and corn silage on adjacent portions of each plot are reported in order to indicate the K_2O and P_2O_5 deficiency of the peat, the data on the flax having been lost. There was no crop produced on the plot which was given phosphate alone.

TABLE 18.—*Phosphoric-acid content of flax (grain) on Radisson plots in 1920*

Plot No.	Fertilization, rate per acre		Ash	P_2O_5 in ash	P_2O_5 in dry matter	Yields per acre		
	Potash salts	Acid phosphate				Clover, timothy hay	Potatoes	Corn silage
	Pounds	Pounds	Per cent	Per cent	Per cent	Tons	Bushels	Tons
1.....	0	400	2.95	39.3	1.16	0.8	23	7.5
2.....	0	0	2.95	36.4	.99	.6	24	7.3
3.....	500	0	2.72	36.4	1.0	1.0	151	12.6
4.....	500	200	3.06	36.9	1.13	1.7	206	20.8
5.....	500	800	3.96	40.1	1.59	2.7	250	26.5
6.....	1,000	800	3.61	36.8	1.33	2.5	304	24.7
7.....	1,000	400	3.25	38.7	1.26	2.8	280	22.4
8.....	500	400	3.11	40.2	1.25	2.3	275	19.7
9.....	250	400	3.50	40.8	1.43	2.1	215	19.1
10.....	125	400	3.42	41.2	1.41	1.7	119	16.5
11.....	0	0	3.02	39.4	1.19	.8	25	5.4
12.....	125	200	2.99	38.4	1.15	2.0	128	10.0

CLOVER AND RAPE GROWN IN BOXES OF COON CREEK PEAT

Late in the winter of 1919–20 medium red clover was planted in wooden boxes, 12 by 12 by 8 inches, and filled with surface peat soil from an unfertilized plot at Coon Creek. One box was fertilized with potash salts, a second with acid phosphate, a third with the combination of potash and phosphate, and a fourth was left untreated. At the same time a parallel set of boxes was planted to dwarf Essex rape. Early in June the plants in the eight boxes were cut off even with the surface, dried, and analyzed, both P_2O_5 and K_2O being determined.

With both crops the P_2O_5 in the dry matter was unaffected by the potash fertilizer alone and increased equally by the phosphate alone and the phosphate with potash (Table 19). The percentage of K_2O in the dry matter was raised by the potash, both used alone and with the phosphate, but by phosphate alone it was much depressed.

TABLE 19.—Composition of red clover and rape grown in plots of peat soil from Coon Creek

Constituent and fertilization	Ash	P ₂ O ₅ in ash	P ₂ O ₅ in dry matter	K ₂ O in ash	K ₂ O in dry matter	Relative yields of dry matter
Red clover:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
None.....	8.80	4.43	0.39	27.27	2.40	100
Potash.....	11.15	3.23	.36	35.96	4.01	100
Phosphate.....	9.25	6.49	.60	16.11	1.49	242
Potash and phosphate.....	10.55	5.88	.62	38.86	4.10	233
Rape:						
None.....	13.10	2.52	.33	4.66	.61	100
Potash.....	16.75	1.79	.30	11.76	1.97	108
Phosphate.....	11.70	7.18	.84	3.33	.39	122
Potash and phosphate.....	12.00	6.66	.80	12.25	1.47	126

CROPS AT FENS

The Fens peat experimental fields lie in the midst of an extensive tamarack swamp in northeastern Minnesota, about 50 miles north of Duluth. They have been operated by the university since the summer of 1915.

The peat at Fens when first cleared of its natural cover of tamaracks, heath plants, and sphagnum moss is unproductive unless treated with phosphate, manured, or burned, by which process phosphate is liberated. It is not benefited by liming, and for some years after breaking shows little or no response to potash applications. However, after from four to six heavy crops of hay have been removed from the phosphate-treated land, potash-starvation suddenly sets in, and after that potash fertilization becomes indispensable for profitable crop production.

TIMOTHY HAY

In the summer of 1916 a timothy-alsike clover mixture was seeded on four plots broken the year before. One, No. 2, received no fertilizer and the others annual applications, No. 1 of potash, No. 3 of phosphate, and No. 4 of phosphate and potash. In 1917 and the three following seasons heavy crops of hay were removed from Nos. 3 and 4, the yields of these each year being much alike. On Nos. 1 and 2 also the yields were alike, very poor on both, the hay coming largely from the so-called "good spots" discussed below. The clover on all the plots had practically disappeared by the end of the third season, leaving a good stand of timothy.

On July 16, 1920, samples of the timothy were taken from the two halves on all four plots. On Nos. 1 and 2 the good spots were carefully avoided in this sampling. The east half of each plot had been treated with nitrate of soda in May, but the effect of this, which had been very noticeable early in June, had practically disappeared by the time of sampling. The analyses are reported in Table 20.

The P₂O₅ was alike on the unfertilized and on the potash-treated plots, but 150 per cent higher on the two that had received phosphate. The K₂O was much alike on the unfertilized and phosphate-treated plots and 60 per cent higher on the two receiving potash. The nitrate applications affected neither the yield nor the content of P₂O₅ and K₂O.

TABLE 20.—*Phosphoric acid and potash in timothy at Fens in 1920*

Plot No.	Fertilization	Ash	P ₂ O ₅ in dry matter	K ₂ O in dry matter	Yield of hay per acre ^a
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Tons</i>
2, west.....	None.....	4.20	0.18	0.98	1.39
2, east.....	Nitrate.....	4.90	.18	1.19	1.28
Average.....		4.55	.18	1.08	1.33
1, west.....	Potash.....	4.77	.20	1.88	.71
1, east.....	Potash and nitrate.....	5.35	.20	1.86	.68
Average.....		5.06	.20	1.87	.69
3, west.....	Phosphate.....	4.53	.53	1.31	3.93
3, east.....	Phosphate and nitrate.....	3.92	.46	.95	4.01
Average.....		4.22	.49	1.13	3.97
4, west.....	Phosphate and potash.....	5.03	.56	1.81	3.39
4, east.....	Phosphate, potash, and nitrate.....	4.54	.54	1.83	3.50
Average.....		4.78	.55	1.82	3.44

^a From 2 cuttings in the season.

It is of interest to note that the K₂O was as high on plot 3, from which heavy crops of hay had been removed in the three preceding years and which at the time was producing a heavy crop, as on the unfertilized plot, No. 2, from which only light crops had been obtained, and that on all plots the K₂O was below what Wagner regards as a safe minimum, 2 per cent. On plot 3, still producing heavily, it had fallen to approximately half that.

SEDGE HAY

On an adjacent field with similar soil a succession of spring fires had killed the trees and burned off the sphagnum-heath plant cover. A good stand of sedge (*Carex*) had occupied the surface but the growth in 1919 had been light. In the spring of 1920 part of this unplowed sedge-covered field was laid out in large plots and these were treated at different rates with 16 per cent acid phosphate. The water level, because of the ditching and tiling on three sides of the field, was well below the surface at all times during the summer, and the fertilization showed little or no effect on the growth of the sedge.

On July 16, 1920, the yield was determined by taking 6 representative square yards from each plot, and the P₂O₅ and K₂O content of each of these 6 square-yard samples was determined (Table 21). The yields and the K₂O content were not appreciably influenced by the phosphate application, but the P₂O₅ content was raised as much as 400 per cent by the heaviest fertilization.

TABLE 21.—*Phosphoric acid and potash in sedge hay from Fens in 1920*

Fertilization, rate per acre	Ash	P ₂ O ₅ in dry matter	K ₂ O in dry matter	Yields of hay per acre
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Tons</i>
None.....	4.35	0.21	1.39	1.09
Acid phosphate, 200 pounds.....	4.73	.36	1.29	.85
Acid phosphate, 400 pounds.....	4.67	.38	1.41	.99
Acid phosphate, 800 pounds.....	4.17	1.01	1.25	.99

In the failure of the sedge to respond to the same fertilization as the timothy the water level may be regarded as the limiting factor, the sedge thriving only when the water is at or a little above the surface. In later experiments at and near both Coon Creek and Golden Valley, as well as elsewhere in Minnesota, the writers have found that these sedges respond to the same fertilization as farm crops and to somewhat the same degree, provided the water level is favorable. Otherwise the fertilization shows no beneficial effect. The edges of plots fertilized in April, when 6 inches of water covered the surface and water continued on the surface until July, were as sharply outlined by their ranker growth as are timothy and clover plots on drained and properly fertilized peat land.

EXPLANATION OF GOOD AND POOR SPOTS

In fields on mineral soils good spots with a much ranker, greener growth of crop are common enough and are most frequently due to the excrement of farm animals.

Reference has already been made to the suspicion of an interchange of fertilizers in the initial applications at Golden Valley and to the analytical evidence that such a suspicion was not justified.

GOOD SPOTS AT GOLDEN VALLEY

There were 32 unfertilized plots in Series I (fig. 1), on the majority of which there was at least one "good spot," on which the growth of the crop was much like that on the corresponding phosphate-treated plot, the plants being tall and well stooled and the yield per unit area many times as heavy as on the rest of the plot. In general, these spots have a rounded outline and range in size from about 2 square yards to a square rod, with the best growth at the center. They persist from season to season, suggesting an unusual supply of phosphate, either from the droppings of farm animals or from shallow burns that occurred before the field was used for experimental purposes.

From two such spots on one of the unfertilized plots samples of winter rye were taken on June 12, 1919, shortly after heads had appeared. At the same time samples were taken both of the typical poor growth on the same unfertilized plots and from the corresponding phosphate-treated plots. The proportion of P_2O_5 in both dry matter and ash (Table 22) from the good spots is similar to that on the phosphate-treated plot, which may be regarded as evidence that these are due to their greater supply of available phosphate, whatever the source of it may have been.

TABLE 22.—*Ash and phosphoric acid in heading plants of winter rye from Golden Valley in 1920*

Fertilization	Growth	Average weight of 100 culms	Ash	P_2O_5 in ash	P_2O_5 in dry matter
		Grams.	Per cent	Per cent	Per cent
None.....	Typical poor growth.....	10	7.88	4.19	0.33
Do.....	"Good spot" No. 1 on same (poor growth) plot.....	80	4.58	9.83	.45
Do.....	"Good spot" No. 2 on same plot.....	88	4.63	10.58	.49
Acid phosphate in 1918 and 1919.....		100	4.90	10.20	.50

GOOD SPOTS AT FENS

At Fens on plot 1, potash only, and plot 2, unfertilized, somewhat similar but much smaller good spots occurred, there being a much taller and heavier growth of hay on these spots than on the rest of the two plots. On July 16, 1920, four of these spots were sampled, and at the same time samples of timothy were secured for analysis from the characteristic unproductive portions of these plots (Table 23). There is in this case nothing in the analyses, except possibly the lowered content of ash, to indicate the explanation of the better growth, but according to Wagner it is not to be regarded as exceptional, on soils very deficient in some one nutrient, for the crop from the plot treated with this nutrient to show no increased percentage of this along with the greatly increased growth. A somewhat similar case has been mentioned above—that of the winter rye at Golden Valley given only 50 pounds per acre of 16 per cent acid phosphate (Table 7).

Wagner, as an example (26, p. 24), describes a field experiment with winter rye in which the straw from an unfertilized plot contained 0.19 per cent P_2O_5 , whereas on one treated with 300 kgm. of acid phosphate and 200 of sodium nitrate per hectare the straw contained only 0.17 per cent. There was, however, an increased yield of straw and grain amounting to 2,550 kgm. per hectare. On the unfertilized plot, because of the shortage of nitrogen, the plants could not make use of the P_2O_5 as well as they could where available nitrogen was supplied them. As a result, they took whatever P_2O_5 the soil offered and deposited it in their leaves and stems without any corresponding growth.

TABLE 23.—*Phosphoric acid and potash in timothy from "good spots" on plots at Fens*

Plot No.	Fertilization	Growth	Ash	P_2O_5 in dry matter	K_2O in dry matter
			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
2	None.....	Poor *.....	4.55	0.18	1.08
2	do.....	Good spot No. 1.....	3.34	.17	1.00
2	do.....	Good spot No. 2.....	3.62	.18	1.10
1	Potash.....	Poor *.....	5.06	.20	1.87
1	do.....	Good spot No. 1.....	4.07	.17	1.50
1	do.....	Good spot No. 2.....	3.90	.17	1.54

* Data are averages from duplicate samples (Table 20).

FLAX AT KARLSTAD IN 1923

At the end of August, 1923, a field of flax adjacent to what is now our Karlstad peat experimental field was examined. It had been fertilized in the spring with treble superphosphate, using a fertilizer spreader, but there were streaks here and there through the field on which the plants were very small. A light frost a week before had injured the bolls on most of the plants and the yield of grain was light. Samples of the small plants, as well as of the common large ones, were collected and analyzed. The dwarfed plants contained 4.81 per cent of ash, 1.29 per cent of K_2O , and 0.18 per cent of P_2O_5 , and the large ones carried 4.84 per cent ash, 1.31 per cent K_2O , and

0.56 per cent P_2O_5 . Evidently these poor streaks were due to the fact that they were missed by the spreader.

Experiments made by the writers in 1924 on the later established adjacent experimental field showed the peat to be as phosphate hungry as that at Golden Valley and to show as little effect from potash applications.

A BENEFICIAL EFFECT OF RYE-STRAW ASH

In 1921 a farmer near Pelican Rapids reported that on the part of a field of peat soil where he had scattered and burned rye straw the following crop was excellent, while elsewhere it was very poor. Concluding that the difference in yield must have been due to the K_2O from the straw, as he knew that comparatively little P_2O_5 is usually carried in the straw, he wanted to purchase a potash fertilizer, but consented to try 200 pounds per acre of acid phosphate on part of the rye which he had already seeded on the same field. At harvest time he reported that the yield on the phosphate-treated portion was many times as heavy as on the rest and sent representative samples of grain in the straw from both parts. These were threshed and analyzed (Table 24). If an analysis of straw samples had been made the year before it would probably have given similar indications—that it was the P_2O_5 and not the K_2O that was deficient, and that although straw carries but little P_2O_5 , this latter had sufficed to cause the marked improvement—akin to the application of 50 pounds per acre of 16 per cent phosphate at Golden Valley in 1919.

TABLE 24.—*Winter rye from Pelican Rapids*

Samples	Ash	P_2O_5	K_2O
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Straw from fertilized portions.....	7.19	0.29	0.39
Straw from unfertilized portions.....	7.45	.16	.78
Grain from fertilized portions.....	1.46	.85	-----
Grain from unfertilized portions.....	1.67	.75	-----

In the same way an analysis of the resulting crop on a partially manured peat field may indicate which constituent in manure, if only one, is responsible for the crop improvement. Illustrations of this may be found in Tables 2 and 4.

DISCUSSION OF ANALYTICAL DATA

So much attention has been given to analytical studies by European investigators that the Minnesota data may most profitably be considered in comparison with some of their findings.⁴

ASH CONTENT

No consistent influence of fertilization upon the proportion of the ash in the crop is shown; sometimes it is increased, at other times it is decreased. This applies to the grains, the cereal straws, and the hays. In general it appears that where the fertilization is light and

* All analytical data of the writers are the averages of concordant duplicate determinations.

limited to the deficient nutrient or nutrients the ash content is lowered in the hays and straws, but where it is limited to a nutrient that is not deficient in the soil the ash content is raised, and where heavy applications are made, even of a deficient nutrient, it is raised.

PHOSPHORIC ACID IN DRY MATTER

The quantity of phosphoric acid was markedly raised in the grains, straws, and hays, except where the fertilization was very light, and a great increase in growth of crop resulted, as with the 50 pounds per acre of acid phosphate at Golden Valley. In such cases much more P_2O_5 was removed in the crop per unit area, although the percentage in the crop was not appreciably higher. The increase was greatest with the straws, from 100 to even 1,000 per cent, and with these the maximum was found when some unfavorable factor limited the growth of the crop, such as a water-logged condition of the soil with the spring grains at Coon Creek in 1920 (Table 15), or too low a water table, as with the sedge at Fens (Table 21). With the grains—rye, oats, barley, flax, and buckwheat—the increase in P_2O_5 content ranged from 10 to 60 per cent in general, but reached 100 per cent with oats and barley and 90 per cent with peas at Golden Valley in the very favorable season of 1918. With the hays of clover, timothy, brome grass, and western rye grass it ranged from 20 to 50 per cent in general, occasionally being higher.

Of the different plants dealt with, where the entire plant was analyzed, rape showed the greatest increase, which might be expected from the studies of Hall, who, as mentioned above, found the related rutabagas the most sensitive test plants for P_2O_5 .

PHOSPHORIC ACID IN THE ASH

The P_2O_5 in the ash varies much like the P_2O_5 in the dry matter, but since the ash content does not regularly vary with the fertilization the P_2O_5 of the dry matter is more significant, and there appears little to be gained from a determination of the amount of the ash or the computation of the percentage of P_2O_5 in it.

EFFECT OF POTASH APPLICATIONS

Where the addition of potash caused an increased yield the percentage of P_2O_5 in the dry matter was lowered, but otherwise it had no effect.

VARIATION IN PHOSPHORIC-ACID CONTENT OF GRAINS

The increase in the percentage of P_2O_5 in the grains, as reported above, is very large in comparison with those found on mineral soils. Wagner emphasizes that the P_2O_5 and K_2O content of the grains of cereals shows but little variation, and Hall (8) found that the P_2O_5 in the ash of barley from the Rothamsted plots showed a variation of less than a fifth, and that with the wheat from the Broadbalk field the greatest difference was less than 6 per cent. With both cereals he found that one extreme was shown on a plot receiving nitrogen only and the other on one given phosphate and potash as well as nitrogen. The P_2O_5 in the ash from the barley on the burned peat at Golden Valley in 1918 was 100 per cent higher than in that from the untreated peat, and with the spring rye it was 40 per cent higher.

Münter, in a study of the wheat crop of successive years at Lauchstadt (15) found still less effect from fertilization than was reported by Hall for the Rothamsted plots.

However, in grain grown on peat, such differences are not to be regarded as unusual. Carl von Feilitzen reported three early experiments with oats (22, p. 96-97, 217) in which, on the plots receiving no phosphate, the percentages of P_2O_5 in the dry matter of the grain were 0.59, 0.37, and 0.37, whereas on the corresponding plots given 600 to 800 kgm. of basic slag per hectare the percentages were, respectively, 0.79, 0.78, and 0.63. A report by H. von Feilitzen (6) of the analyses of over 800 samples of different crops grown on peat shows what great variations may be expected.

VARIATION IN PHOSPHORIC-ACID CONTENT OF CEREAL STRAW

The effect of fertilization upon the P_2O_5 content of the straw was much greater even than upon that of the grains, but that this is not to be regarded as unusual may again be illustrated from the Rothamsted data, reported by Hall (8), taking the wheat from the same two plots referred to above. The percentages of P_2O_5 in the ash from the straw of these were 2.50 and 3.73 in 1852, 1.73 and 2.78 in 1863, and 2.12 and 3.82 for the crops of the 10-year period 1882-1891. Münter, at Lauchstadt, however, found but little effect upon the straw of wheat (15).

The great effect of fertilization upon the P_2O_5 content of the buckwheat straw at Coon Creek (Table 14) is not approached by any previously published data found by the writers, except in the case of pot experiments, where, on the one hand, an abnormal hunger may be induced by entirely withholding one of the nutrients, and, on the other hand, it may be appeased by applying this nutrient in excessive quantities.

EXTREME VARIATIONS OBTAINABLE IN POT EXPERIMENTS

In the field we should expect to find but seldom the differences that may readily be induced in pot experiments. An excellent illustration of the extreme effect of differences in fertilization that may be obtained in pot experiments is furnished by a recent study by Pfeiffer et al. (19, p. 8, 12, 17), in which oats was used as the test crop and harvested when the grain was in the early milk. Some of their data have been assembled in Table 25. They used a sand practically devoid of available nutrients, 16 kgm. to the pot, and in each series of experiments supplied liberal quantities of all the nutrients except the one whose influence was being studied, the nitrogen, potassium, and phosphorus being supplied in the form of ammonium nitrate, potassium sulphate, and acid calcium phosphate. The results of these investigations accord with the field data obtained in the Minnesota experiments. With extreme hunger of a nutrient the first or lightest applications cause a great increase in the yield, but very little effect upon the percentage of this nutrient in the crop. With increasing quantities the yields increase less markedly and the nutrient content more rapidly. Finally, when the applications have become so heavy that the yield is almost at its maximum, the proportion of the nutrient rises rapidly. This is most marked in the case of the P_2O_5 and least with the nitrogen.

TABLE 25.—*Variations in composition of oats caused by differences in fertilization, as found in pot experiments by Pfeiffer, Simmermacher, and Rippel (19)*

Experiment No.	Phosphoric-acid variable			Potash variable			Nitrogen variable		
	Added P_2O_5	Crop yield	P_2O_5 in crop	Added K_2O	Crop yield	K_2O in crop	Added N	Crop yield	N in crop
	Grams	Grams	Per cent	Grams	Grams	Per cent	Grams	Grams	Per cent
1-----	0.00	14	0.12	0.0	4	0.55	0.00	7	0.61
2-----	.05	36	.11	.1	23	.58	.2	33	.51
3-----	.10	61	.13	.2	29	.77	.4	56	.56
4-----	.20	117	.14	.4	66	.61	.6	90	.52
5-----	.30	150	.15	.6	92	.66	.8	110	.59
6-----	.45	169	.19	.9	103	.77	1.8	181	.87
7-----	.60	189	.23	1.2	122	.85	2.1	189	.91
8-----	.80	199	.29	1.5	115	1.12	2.4	197	1.00
9-----	1.20	203	.36	2.0	143	1.25	2.7	200	1.04
10-----	2.00	216	.55	3.0	144	1.76	3.0	209	1.15
11-----	3.50	215	.80	4.5	132	2.74	4.0	226	1.38

Variations in the P_2O_5 content of the crops on the Minnesota experimental peat fields have been almost as extreme as those found by Pfeiffer (19) in his pot experiments.

PHOSPHORIC-ACID CONTENT OF HAY CROPS

On peat probably the most interest attaches to the composition of the hay crops. According to Wagner's rules, which place at 0.65 the P_2O_5 content of hay crops from land so well supplied with available phosphate that further applications would not increase the yields, the analyses would suggest that the peat at Golden Valley—in the untreated condition so poor in available phosphate that it gave hay with less than 0.25 per cent phosphoric acid, and even when treated with phosphate less than 0.40 per cent—might well have been given a much heavier initial application of phosphate, as is the custom in continental Europe. At Coon Creek, where the phosphate hunger is far less pronounced, the P_2O_5 content of the hay is higher on both the fertilized and unfertilized peat, but even on the former it is still somewhat below 0.65 per cent.

Tacke, from a study of the composition of the ash of hay from many German bogs and marshes (23), concluded that Wagner's values were satisfactory for meadows on peat soils, Tacke having found as the averages for all the analyzed samples of hay from the Bremen peat experimental station 0.67 per cent P_2O_5 and 2.04 per cent K_2O (24, p. 18). The averages for 40 samples of hay from meadows on peat soils fertilized with potash and phosphate were 0.70 per cent of P_2O_5 and 2.49 per cent of K_2O , on the basis of 16 per cent of water in the hay (14, p. 50).

H. von Feilitzen (5, p. 265) disagreed with Tacke, reporting analyses from a large number of Swedish meadows on both high moors and low moors, in none of which the P_2O_5 was found to be as high as 0.65 per cent. The averages of all the analyses of hay made at Jonkoping up to 1909 showed 0.40 per cent for P_2O_5 and 1.67 per cent for K_2O for the high moors (low-lime peats) and 0.35 per cent P_2O_5 and 1.43 per cent K_2O for the low moors (nearly all of the high-lime type). The yields on the meadows, he believed, would not have been increased by heavier fertilization with phosphate and potash;

and the saturation values, he concludes, are lower on the Swedish than on the German bogs.

Tacke does not accept this conclusion as proven, although he points out that the average P_2O_5 content of the hay from German unfertilized meadows on peat soils is higher than that of the Swedish meadows fertilized with both phosphate and potash (24, p. 313). In the same article he calls attention to the great variation in phosphate and potash shown by different hay plants grown under exactly the same conditions, the P_2O_5 ranging from 0.88 to 1.42 per cent and the K_2O from 1.16 to 2.39 per cent, and to the influence of the weather of the crop season, both these constituents averaging lower in dry than in wet seasons, as in the latter the absorption of mineral matter can proceed uninterruptedly (24, p. 16).

H. von Feilitzen reports the P_2O_5 content of three hay grasses taken from a meadow fertilized heavily enough with basic slag and kainite to give maximum yields. In the timothy he found 0.35 per cent P_2O_5 , in the reed canary grass (*Phalaris arundinacea*) 0.40 per cent, and in meadow foxtail (*Alopecurus pratensis*) only 0.23 per cent (5, p. 341-344).

MEADOW STUDIES OF MAYR AND AHR

The most critical experimental study of the applicability of Wagner's conclusions is probably that reported by Mayr and Ahr (13), who in their investigations employed six meadows—two on peat, two on peaty soils (with a coating of only a few inches of peat before plowing), and two on mineral soils. In 1910, before laying out the meadows in plots for the fertilizer experiments, samples of both hay (1) and aftermath (2) were taken from each of the six, and in each of the five consecutive years 1912, 1913, 1914, 1915, and 1916 samples of both hay and aftermath were analyzed. For the crops of each of the first four of these years there were annual applications of fertilizers—78 kgms. of P_2O_5 and 100 kgms. of K_2O per hectare—but for the last year no application was made and only the residues of the preceding years came into play. The crops of 1910 showed an extreme range in both constituents, the P_2O_5 varying between 0.44 and 0.92 per cent and the K_2O between 1.09 and 4.20 per cent. The highest values were shown by a very productive meadow (Pfaffanger) on mineral soil, the first cutting from which in 1910 carried 0.86 per cent P_2O_5 and 3.90 per cent K_2O ; the lowest values were found in a very unproductive meadow (Waschanger) on peat, with only 0.48 per cent P_2O_5 and 1.13 per cent K_2O . From Wagner's conclusions no increase from fertilization was to have been expected in the case of the former, but certainly a marked response to potash, and very probably to phosphate also, in the case of the Waschanger meadow. However, the subsequent experiments did not fully confirm these forecasts. In the case of the productive meadow in each of the five seasons the applications of potash with phosphate increased the yield from 3 to 11 per cent, with an average of 7, while for the five seasons (1912-1916) on the unproductive meadow the average yield was increased only 18 per cent by potash alone, 5 per cent by phosphate alone, and 36 per cent by the two together. The content of P_2O_5 and K_2O of the hay from the former was quite inferior to that

of 1910, the averages for all the cuttings obtained in the five seasons being 0.65 and 1.84 per cent on the former and on the latter 0.39 and 1.36.

	Pfaffanger meadow		Waschanger meadow	
	Per cent of P_2O_5	Per cent of K_2O	Per cent of P_2O_5	Per cent of K_2O
First cutting in 1910.....	0.86	3.90	0.48	1.13
Average for all cuttings, 1912-1916.....	.65	1.84	.39	1.36

The variations in P_2O_5 and K_2O from crop to crop on the Waschanger field (Table 26) well illustrate the fluctuations on all six meadows. When data from the same cutting in any season are compared it is seen that the application of phosphate has in every case markedly increased the P_2O_5 content of the crop and the application of potash has increased the K_2O content. From crop to crop, however, there is a marked variation in the percentage, as though it had been affected by the weather of the season. Thus the P_2O_5 was exceptionally high in the aftermath on all plots in 1914 and exceptionally low in the aftermath of the following year. In 1914 it was higher on the plots given no phosphate than in 1915 on those that had received phosphate. There were less extreme fluctuations from season to season in the potash content. In the case of the plots receiving potash fertilizer there was a lower percentage of K_2O in the second crop than in the first, except in 1914. There was no such relationship in the P_2O_5 content of the crops from the plots receiving phosphate.

TABLE 26.—*Phosphoric acid and potash in hay, two cuttings (1 and 2) in each season, on experimental field at Waschanger in Austria, from report of Mayr and Ahr (13)*

Treatment	1910		1912		1913		1914		1915		1916		Average, 1912-1916		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	Both
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Phosphoric acid in dry hay:															
With phosphate.....	0.49	0.55	0.62	0.69	0.65	0.79	0.51	0.28	0.63	0.66	0.58	0.59	0.58	0.59	0.58
Without fertilizer.....	0.48	.44	.31	.35	.45	.46	.41	.49	.30	.27	.39	.44	.37	.40	.39
With potash.....44	.44	.45	.42	.36	.53	.35	.26	.37	.44	.39	.42	.41
With phosphate and potash.....49	.56	.61	.73	.60	.74	.49	.33	.56	.62	.55	.60	.57
Potash in dry hay:															
With phosphate.....	1.45	1.23	1.43	1.34	1.34	1.56	1.38	1.28	1.29	1.21	1.35	1.35	1.35	1.35	1.35
Without fertilizer.....	1.13	1.09	1.52	1.24	1.30	1.27	1.42	1.66	1.12	1.38	1.39	1.18	1.38	1.35	1.36
With potash.....	2.02	1.58	1.86	1.75	2.06	2.13	2.47	2.16	1.92	1.64	2.06	1.85	1.96
With phosphate and potash.....	1.96	1.55	1.77	1.59	2.00	2.00	2.30	2.10	1.68	1.44	1.94	1.74	1.84

Mayr and Ahr (13) regard the soundness of Wagner's conclusions as generally confirmed by their work, and consider the method of ash analysis worthy of a regular place in the practical control of the fertilization of meadows and pastures. However, they insist that the chemical composition of the crop must be regularly supple-

mented by the determination of the botanical composition, at least as to the proportions by weight of clovers and other legumes and also of weeds. The legumes are generally richer in P_2O_5 and poorer in K_2O than the grasses. Mayr and Ahr consider the method less valuable in the case of phosphoric acid than in that of potash, concluding that "at the present time, for deciding upon the urgency of potash fertilization of meadows and the degree of this, there is no better method than the chemical and botanical investigation of a properly taken sample from the first and second cutting, determining the proportion by weight and the potash content of the main groups of plants." Both diagnosis and prognosis are made more reliable by the analyses of samples from both the first cutting and the aftermath. Instead of taking the samples for analysis from the cured hay after the crop is gathered, 4 to 5 kgm. of green material should be carefully collected at the time of mowing and at once dried artificially without being exposed to the possibility of loss.

RELATION OF PHOSPHORIC-ACID CONTENT OF CROPS TO ANIMAL NUTRITION

The palatability and nutritive value of tame hay from peat lands has from time to time been suspected, and nutrition deficiencies have been attributed to its use. The situation up to 1912 has been well reviewed by both Bersch (4, p. 281-283) and Fleischer (7, p. 59-61), who deal with the "licking disease" (Lecksucht) of cattle, which had been observed in many scattered districts when the hay from peat meadows was fed to calves. In some cases it was so serious that it might prevent the raising of calves. The disease manifests itself chiefly in a marked tendency to gnaw and lick various indigestible objects—mortar, stones, wood, twine, pieces of cloth, hair, animal excrement. The German Central Moor Commission, which early devoted much attention to this problem, had Ostertag (18) make a careful study of it. Although Ostertag did not find the cause, he concluded that the disease-producing hay was harmless with horses; by steaming, the injuriousness could be so far removed that calves could be fed with it for a long time without developing symptoms of the disease, and by curing it so as to get "brown" hay⁵ all harmful qualities would disappear. If the first cutting on such meadows was removed very early—before the blooming of the grasses—it was harmless and very palatable, but the aftermath could not be made harmless in this manner. Fertilization with sodium nitrate lessened the injuriousness of the hay. The same meadows when pastured caused no ill effects, and when cattle affected by the hay were pastured on the same meadows from which the hay had been obtained they recovered unless the disease was already too far advanced. Giving of medicines and concentrates without pasturing was not effective; neither was the giving of sodium salts and lime. Bersch (4) states that Baumann and Soxhlet found the disease-producing hay poorer in an organic phosphorous-containing compound, though not in the P_2O_5 content of the ash.

After reviewing the evidence Bersch concluded that the hay from tame meadows on peat soil in general is just as palatable and nutri-

⁵ A detailed description of the making of brown hay is to be found in *Die Kultur der Wiesen*, by W. Strecker (21, p. 427-429).

tious as that from meadows on mineral soils and that no difference at all exists which under all circumstances would cause the peat-meadow hay to have a lower value.

Farmers in the vicinity of Golden Valley in 1919 mentioned an abnormal appetite shown by their cattle, similar to that in Bersch's (4) description, which they observed especially during the late winter and spring. Since these animals were fed during the winter largely upon the hay from the unbroken peat lands, and this consisted chiefly of sedges, samples of which from the Golden Valley fields had been found very low in phosphoric acid—0.26 per cent in the spring of 1921—it was suggested to the farmers who were buying treble superphosphate that they add some of this to the feeds. Many did so, and reported favorable results.

McClendon (11) in 1920 having mentioned that, according to Voegtlin and Myers, both antineuritic and fat-soluble vitamins of wheat and corn products run parallel to the phosphorus content, it was suggested that he try a feeding experiment with the grains from Golden Valley whose analyses have been given above (Tables 3, 6, and 7), but of which the quantities were very small. McClendon and Henry (12) fed some of the oats and barley to rats for 65 days and found that the rats given the grain richer in P_2O_5 made the more rapid growth. Two lots of each cereal were used, the oats being from the unfertilized peat, with 0.52 per cent P_2O_5 , and the adjacent mineral soil, with 1.10 per cent P_2O_5 , while the barley was from the untreated peat, with 0.50 per cent P_2O_5 , and from the burned peat, with 1.06 per cent P_2O_5 . The very limited quantities of the grains that had been saved prevented any more extensive experiment at that time.

The great interest that has arisen in the last few years in the use of mineral supplements in livestock feeding, the extensive and rapid development of clover-timothy meadows on peat land in Minnesota and the frequently expressed suspicion as to the feeding quality of the well-cured clover and timothy-clover hay grown upon the well-fertilized peat meadows have redirected the attention of the present writers to this subject. If the desired minimum percentage of P_2O_5 in the cured hay, from the standpoint of the animal feeder, should be established at a value much above the Swedish average of 0.40 per cent, the writers' recommendation as to phosphate fertilization of peat meadows will need modification. These were based upon what their experiments showed to be the most economical applications for the production of hay—rates that will give a little below the maximum obtainable yields—the final increases in the field, as in Pfeiffer's (19) pot experiments described above, being brought about only by very heavy applications (Table 25).

In the case of a large tract of sedge-covered peat near Coon Creek, broken, fertilized, and seeded in the spring and early summer of 1925, fifty $\frac{1}{16}$ -acre plots were seeded with a mixture of alsike red clover, and timothy in May, and in early June fertilized with treble superphosphate and muriate of potash in varying amounts. The hay was cut on September 18. The application of 100 pounds of the phosphate and 200 pounds of the potash (44 pounds of P_2O_5 and 99 pounds of K_2O) per acre gave almost as high a yield—2.2 tons per acre—as an application in the same proportions but at double the

rate. Hence the lighter application would be distinctly more profitable to the owners of the meadow, who keep no stock but sell the hay. The unfertilized plots yielded 1.1 tons per acre with 0.31 per cent P_2O_5 and 1.35 per cent K_2O . The crop from the lighter fertilization contained 0.38 per cent P_2O_5 and 1.69 per cent K_2O while that from the heavier application carried 0.54 per cent P_2O_5 and 3.19 per cent K_2O .

The 2.20 tons of hay from 1 acre in the case of the more heavily fertilized peat carried 7.04 pounds more P_2O_5 than the more lightly fertilized, but to obtain this increase 44 pounds additional P_2O_5 was applied in the fertilizer. If it should prove that additional P_2O_5 in the feed is desirable and a pound of P_2O_5 given in the form of bone meal or some other carrier of calcium phosphate should prove as effective as an equal amount in the hay, it would be much more economical to so supply it rather than to add many times as much to the soil. If some feed richer in P_2O_5 than hay were included in the ration, this would affect the case.

The very favorable views on the feeding value of hay from peat meadows expressed by Bersch may be based largely on observations on the use of hay from meadows given very heavy initial applications of phosphate, such as he recommends (1, p. 43; 4, p. 165), 110 to 270 pounds per acre of P_2O_5 in the first year and 55 to 160 in the second, making 165 to 435 pounds in the first two seasons, equivalent to 1,030 to 2,720 pounds of 16 per cent acid phosphate. The writers, on the other hand, find about 300 pounds in the first year and 150 in the second to be generally the most profitable. The possibility of the "licking disease" being connected with a low P_2O_5 content of the hay has not been overlooked in Germany and Austria.

If it should be found desirable to use a heavy initial application of phosphate in order to increase the P_2O_5 content of the crops, the increased expense occasioned by this would usually not be prohibitive where the owner did not intend to soon sell his improved peat land, as there is no leaching out of the applied P_2O_5 in the case of the high-lime peat soils.

HAY FROM PHOSPHATE-HUNGRY MINERAL SOILS

Phosphate hunger in Minnesota, although found in its most extreme development in some of the peat soils, is by no means confined to these, being very common in about a third of the State—on the dark prairie soils of the western and southwestern counties. West of a line drawn northwestward from Albert Lea, near the Iowa boundary, to Roseau, near the Canadian border, it would appear from the field experiments of the Division of Soils that on at least one-third of the fields acid phosphate, either in the form of the ordinary 16 or 20 per cent product or of the concentrated 44 per cent material known as treble superphosphate, can be used with profit on alfalfa and the clovers, the crops most responsive to phosphate fertilizers. Wheat and barley show less response, and oats and corn still less. The phosphate hunger is not limited to any particular soil types, although, on the whole, the naturally poorly drained lands show the most marked response. It appears on the fields of the most intelligently managed livestock farms, as well as on the unmanured fields of the tenant farms. The use of a short

rotation, including clover, the feeding on the farm of all the crops grown on it, and the prompt return of the manure to the fields, with the sale of chiefly young beef animals, seems rather to hasten than to delay the appearance of phosphate deficiency in the soil.

With P. R. McMiller the senior author tried applications of 100 pounds per acre of treble superphosphate on about 30 fields of red clover and alfalfa in Jackson County in 1923 and 1924. Somewhat more than 60 per cent of these showed a marked response to the fertilizer. To determine whether the analysis of the crops might indicate a P_2O_5 deficiency in the soils, analyses were made of samples of hay from 10 of the fields, including those that had shown the greatest response (fig. 8). In each field the hay was carefully gathered from 4 representative square yards on the fertilized plot and from a similar number on the adjacent unfertilized portion of the field. The samples were dried, weighed, and later analyzed (Table 27). The P_2O_5 content of the hay from the unfertilized land ranged from 0.38 to 0.57 per cent, with an average of 0.49 per cent. That in the hay from the fertilized plots ranged from 0.52 to 0.89 per cent, with an average of 0.74 per cent. Neither the quantity of P_2O_5 in the hay from the unfertilized land nor the increase caused by fertilization is distinctly related to the degree of phosphate hunger as indicated by the increase in yield.

Crop analysis does not appear promising as a practical method of detecting the phosphate-hungry fields on these prairie soils and is not to be compared in desirability with the use of small-plot trials, which may be satisfactorily carried out by any intelligent and interested farmer. A dollar's worth of phosphate is sufficient for twenty $\frac{1}{10}$ -acre plots—usually enough for a whole farm.

The P_2O_5 content of these Jackson County hays, even from the unfertilized land, is high compared with that in clover and timothy hay from the peat experimental fields. The analyses of some samples from Golden Valley and Coon Creek, collected by G. H. Nesom in 1922 and 1923, are reported in Tables 28 and 29.

At Golden Valley in 1922 the hay from the plots receiving phosphate contained less than 0.40 per cent P_2O_5 . The yield was so greatly increased by the phosphate, however, that from eight to twelve times as much P_2O_5 per unit area was removed in the crop. In 1923, with the lighter yields, the P_2O_5 content was higher in the hay from both unfertilized and fertilized plots.

At Coon Creek in 1922 the timothy from the plots receiving phosphate and potash contained but little more P_2O_5 than that from unfertilized mineral soil in Jackson County (No. 7 in Table 27).

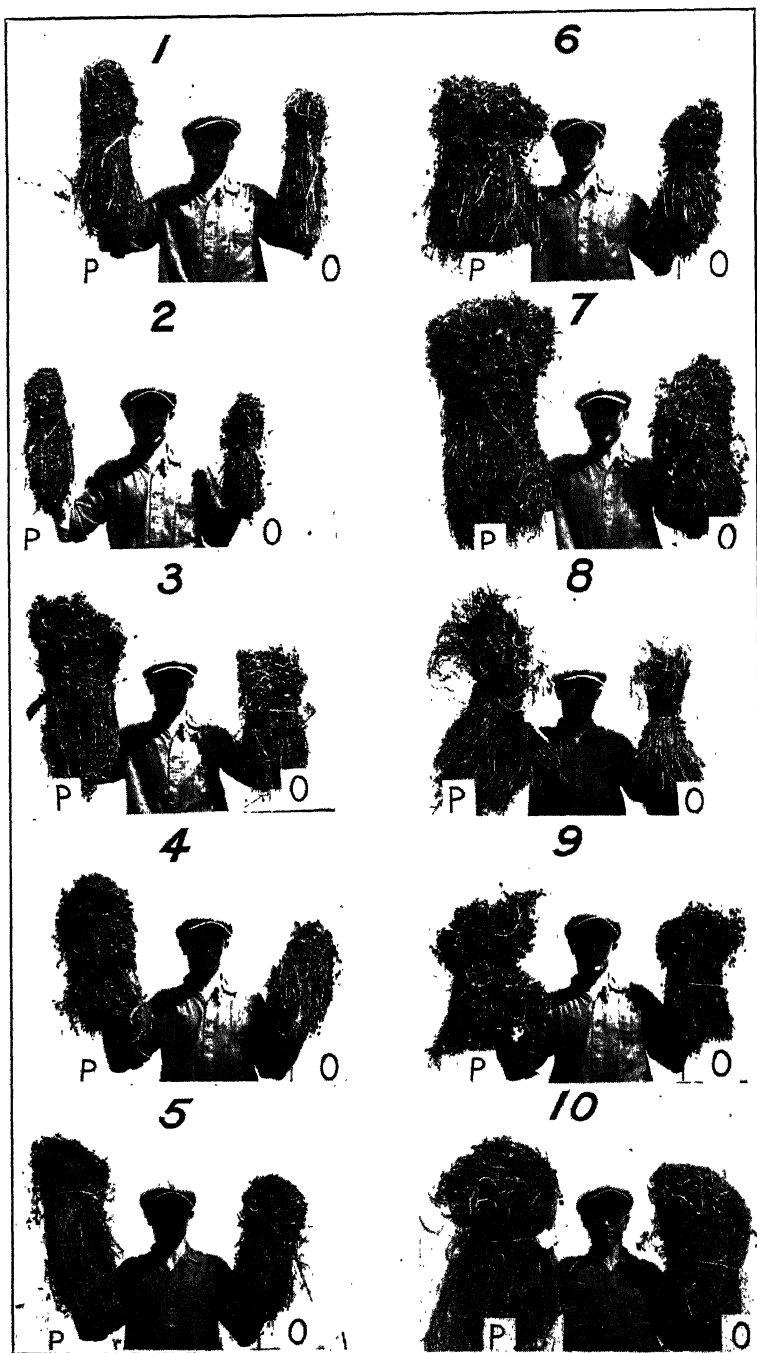


FIG. 8.—Hay samples from mineral soil in Jackson County in 1923. Each bundle is from 4 square yards, the right in each of the 10 pictures being from unfertilized land, and therefore smaller, and the left from the phosphate-treated plot. The analyses are reported in Table 27

TABLE 27.—*Phosphoric-acid content of hay samples from clover-timothy fields on mineral soil in Jackson County (first cutting in 1923)*

Field		Proportion of clover in hay	Relative yield with phosphate ^a	P ₂ O ₅ in dry matter			Ash			P ₂ O ₅ in ash		
No.	Owner			With no fertilizer	With phosphate	Relative amount with phosphate ^b	With no fertilizer	With phosphate	Relative amount with phosphate ^b	With no fertilizer	With phosphate	Relative amount with phosphate ^b
		<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
1	J. C.	80	250	0.55	0.75	136	8.56	10.60	124	6.46	7.08	109
2	C. T.	80	200	.56	.77	137	10.38	12.39	119	5.39	6.21	115
3	E. M.	90	200	.48	.67	139	8.95	10.30	115	5.36	6.50	121
4	A. G.	100	200	.49	.78	159	9.43	11.29	119	5.20	6.91	133
5	A. S.	90	200	.49	.80	163	10.46	11.73	112	4.68	6.82	145
6	W. S.	110	200	.51	.89	174	9.42	10.49	111	5.41	8.98	156
7	G. G.	100	150	.57	.84	147	11.12	10.20	91	5.13	8.23	160
8	C. F.	0	150	.38	.52	137	7.09	8.52	120	6.13	7.19	117
9	S. Ha	100	125	.49	.78	159	12.91	13.42	104	5.19	6.63	127
10	S. Ho	60	110	.42	.65	155	9.42	9.43	100	4.46	6.89	154
Average		81	178	.49	.74	150	9.77	10.83	111	5.34	7.14	133

^a Yield on unfertilized plot is placed at 100.^b Per cent in sample from unfertilized plot is placed at 100.TABLE 28.—*Phosphoric-acid content of red-clover hay on Golden Valley peat experimental fields in 1922 and 1923*

Fertilization	Plot	P ₂ O ₅ in dry matter		Ash in dry matter		Yield of hay per acre	
		1922	1923	1922	1923	1922	1923
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Tons</i>	<i>Tons</i>
None	C-XI	0.26	0.36	9.41	8.09	0.43	0.16
Potash	B-XI	.25	.36	9.88	9.92	.46	.04
Phosphate	C-XII ^a	.38	.56	6.92	7.43	3.94	.85
Phosphate and potash	B-XII ^b	.29	.51	9.08	7.53	3.75	1.09
Heavily burned in 1918	C-VIII						

^a Data for 1923 from plot B-XII.^b Data for 1923 from plot B-XIV.TABLE 29.—*Phosphoric-acid content of timothy hay on Coon Creek experimental fields in 1922*

Fertilization	Plot	P ₂ O ₅ in dry matter	Ash in dry matter	Yield of hay per acre
		<i>Per cent</i>	<i>Per cent</i>	<i>Tons</i>
None	B-III-1	0.23	3.79	0.67
Do.	C-III-1	.23	3.83	.85
Average		.23	3.81	.76
Potash	B-II-4	.24	4.51	2.42
Do.	C-II-4	.21	4.35	1.33
Average		.22	4.43	1.88
Phosphate and potash	B-II-5	.42	5.13	3.90
Do.	C-II-5	.41	4.33	3.54
Average		.41	4.73	3.72

SUMMARY

With grain, straw, and hay from unfertilized and adjacent fertilized plots determinations were made of the ash and P_2O_5 content, and in some cases of the K_2O also.

The ash content of the crop was in some cases increased but in other cases decreased by fertilization. In general, with the cereal straws and the hays, where the fertilization was limited to the deficient nutrient or nutrients, the ash content was lowered when the rate of application was low, but raised when it was high, and where the fertilization was limited to a nutrient that was not deficient the ash content was raised.

The P_2O_5 content of the hays, straws, and even grains grown on phosphate-hungry peats was low, this being most marked on the straws and least in the grains. It was greatly increased by phosphate fertilization, most in the straws and least in the grains, except where the application was very light and a greatly increased yield resulted. It was greatest where some unfavorable factor, other than the supply of nutrients, caused a low yield. With the straws the increase in some cases amounted to several hundred per cent, with the hays from 20 to 50 per cent, and even higher, and with the grains usually between 10 and 60 per cent. The P_2O_5 content of a hay crop grown on a plot receiving the same fertilization each year is so affected by weather conditions that in one season it may be as high on an unfertilized plot as in the next on an adjacent phosphate-treated plot. The P_2O_5 determination has most significance when comparisons are made between fields or plots of a crop in the same locality and in the same season and with hay crops of the same cutting. In the case of hay crops it is important that the analyses be made of the separated important components instead of the mixed samples containing unknown proportions of different plants.

The percentage of P_2O_5 in the ash varies much like that in the dry matter, but as the ash does not vary regularly with the fertilization the proportion of P_2O_5 in the dry matter is more significant than is that in the ash, and there appears little to be gained from the computation of the percentage of P_2O_5 in the ash.

Potash applications affect the P_2O_5 content of the crop as they affect the yields. When potash increases the yield it lowers the percentage of P_2O_5 in the crop. In some cases an application of a mixture of potash and phosphate will greatly increase the yield of grain without raising the proportion of P_2O_5 . Where a potash application does not increase the yield it increases the percentage of ash, and hence lowers the proportion of P_2O_5 .

The extreme variations in P_2O_5 content found, although much greater than those reported for crops grown on mineral soils, are not wider than those found for crops on peat lands in Sweden, Germany, and Austria, nor wider than those found in pot experiments.

The analysis of the crops is very useful in detecting mistakes in the application of fertilizers—either the substitution of one nutrient for another or the omission of one from an intended mixture. It also will frequently serve to explain the cause of productive spots and areas on unfertilized fields or plots of a peat that is elsewhere unproductive.

Definite limiting values for the P_2O_5 content of a crop, both an upper one, indicating that the phosphate supply in the soil is adequate for approximately maximum yields, and a lower one, indicating that heavier phosphate fertilization is practically certain to cause a great increase in yield, are difficult to place, at least for peat soils, since values that would apply for seasons or localities with favorable weather conditions, and consequent heavy yields, are likely to be so high as to require unprofitably heavy fertilization.

If animal nutrition investigations of hays from peat lands should lead to the conclusion that a certain minimum content of P_2O_5 in the hay is desirable from the standpoint of the feeder, it can be secured by a heavy initial phosphate application, but with a decreased profit for those peat-meadow owners who produce hay for sale.

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THE PREPARATION AND EFFECTIVENESS OF BASIC COPPER SULPHATES FOR FUNGICIDAL PURPOSES¹

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INTRODUCTION

The practice of treating certain plants with copper fungicides has created a demand for a ready-made product to be used in place of Bordeaux mixture, since the latter is unstable and somewhat difficult to prepare. Numerous preparations, both wet and dry, have been offered as substitutes. Most of these have proved rather unsatisfactory. This led the writers to undertake further study of the subject with a view to producing a powder of good physical properties that might be applied either as a spray or as a dust. Since basic copper sulphates have generally been found preferable to basic carbonates, attention was turned largely to these.

EARLIER INVESTIGATIONS

Basic copper sulphates are insoluble copper compounds resulting from the action of alkali or alkaline earth hydroxides or carbonates, short of saturation, upon copper sulphate, although other precipitants are occasionally employed. Pickering (10, p. 1982)² has shown that different hydroxides react in substantially the same way, but that the color of the basic sulphate varies with the kind and quantity of alkali used and with the degree of hydration through different shades of green and blue. Some writers (8, p. 261) question, however, whether the precipitates are definite compounds or mixtures—i. e., solid solutions—but the following appear sufficiently established to warrant consideration: Dibasic sulphate, $3\text{CuO} \cdot \text{SO}_3 \cdot n\text{H}_2\text{O}$; tribasic sulphate, $4\text{CuO} \cdot \text{SO}_3 \cdot n\text{H}_2\text{O}$; tetrabasic sulphate, $5\text{CuO} \cdot \text{SO}_3 \cdot n\text{H}_2\text{O}$; nonobasic sulphate, $10\text{CuO} \cdot \text{SO}_3 \cdot n\text{H}_2\text{O}$; and pentabasic disulphate, $7\text{CuO} \cdot 2\text{SO}_3 \cdot n\text{H}_2\text{O}$.

The dibasic sulphate is a green or greenish-blue precipitate obtained by boiling a neutral solution of copper sulphate (9, p. 181; 12, p. 1855); by adding ammonia insufficient for complete precipitation (1; 18; 15, p. 205); by adding insufficient ammonia to a boiling solution (15, p. 205) or by adding insufficient alkali carbonate to a boiling solution of copper sulphate (15, p. 206) with careful washing and drying. The degrees of hydration reported are 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, and 3 mols.

The tribasic sulphate is a green, greenish-blue, or blue precipitate obtained by adding fixed alkalies, sufficient for complete precipitation (0.75 mol.), to a solution of copper sulphate (16; 10, p. 1982; 12, p. 1855); by adding alkaline earth hydroxides to complete precipitation (10, p. 1982); by adding insufficient fixed alkalies (13,

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² Reference is made by number (italic) to "Literature cited," p. 750.

p. 34; 5); by adding insufficient ammonia (19); by adding insufficient sodium carbonate (16); by adding sodium acetate to copper sulphate (9, *p.* 182); by adding sodium silicate to copper sulphate (17) or by adding copper sulphate to sodium acetate (4, *p.* 24-37; 7, *p.* 212). The hydrations reported are 2, 3, 3½, and 4 mols. The minerals brochantite and langite are of rhombic structure and are said to be tribasic copper sulphate with 3 and 4 mols. of water respectively.

Tetrabasic sulphate is a greenish-blue or blue precipitate obtained by adding alkali or alkaline earth hydroxides, sufficient for initial alkalinity (0.8 mol.), to copper sulphate (16; 10, *p.* 1982; 12, *p.* 1855). The hydration reported is 6 mols. With any additional alkali the mixture commences to yield cupric oxide on boiling; and with dextrose, cuprous oxide (12, *p.* 1852, 1855).

Nonobasic sulphate is generally a full blue, bulky precipitate obtained by adding alkali or alkaline earth hydroxides, sufficient for permanent alkalinity (0.9 mol.), to copper sulphate (10, *p.* 1982). Nonobasic sulphate is unstable, turns black on heating, with the formation of cupric oxide, and with dextrose precipitates cuprous oxide in the cold (12, *p.* 1852, 1855).

Succeeding the nonobasic sulphate is a double sulphate, 10 CuO·SO₃·3CaO, a blue, bulky precipitate obtained by adding copper sulphate to a like weight of calcium oxide as hydroxide (11, *p.* 1991, 1997, 2000). Whether other alkaline earth hydroxides react in a similar manner has not been determined. The double sulphate is stable on boiling; but a portion of the copper dissolves in dextrose, in the cold, to a purple solution and precipitates cuprous oxide (12, *p.* 1852, 1855).

A green or greenish-blue pentabasic disulphate is occasionally mentioned and is said to be obtained by adding an alkali hydroxide to an excess of copper sulphate (13, *p.* 34); by adding slowly a concentrated solution of an alkali carbonate to a boiling concentrated solution of copper sulphate, with constant agitation (6); by boiling copper sulphate and potassium sulphate (2, *p.* 477; 3, *p.* 223; 15, *p.* 204-205), or by the action of porous limestone on copper sulphate (8, *p.* 264). The hydrations reported are 5, 6, and 7 mols. Some writers claim brochantite is pentabasic disulphate with 5 molecules of water.

PRELIMINARY WORK

The physical properties of tribasic copper sulphate, precipitated by alkali or alkaline earth hydroxides or carbonates and dried, are often poorly adapted for fungicidal purposes, being dense and even gritty after careful grinding. The same is substantially true of tetrabasic sulphate, although it is rather more bulky as a rule; and nonobasic sulphate is generally too unstable to withstand drying without more or less decomposition. The solubility and basicity of the various precipitants and the solubility of the by-products are a matter of record and the leavening effect of a carbonate might be anticipated, but the necessary physical attributes of the basic sulphate could not be deduced from the work previously cited.

Of the hydroxides the alkaline earths were more promising precipitants than the alkalis on account of their lower activity and were largely employed; 0.8 mol. or more with 0 to 300 c. c. of water

and 7.49 gm. of copper sulphate in 100 to 300 c. c. of solution were mixed, thoroughly agitated, filtered, and dried. Heat was applied in some instances to facilitate the reaction. The purity of the basic copper sulphate is affected by the presence of insoluble by-products except in the case of magnesium. Eight-tenths mol. of barium hydroxide, $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, gave a neutral mixture at room temperature, but careful stirring was required to assure complete precipitation of the copper, and the resultant blue precipitate when dried was hard and unsatisfactory for spray purposes. The precipitate from a hot solution is generally unstable and turns black. A paste prepared by one of the writers (Dunbar), by adding a saturated solution of barium hydroxide to a dilute solution of copper sulphate to slight alkalinity and removing the excess water by filtration, kept in excellent condition for months. The paste gave fair suspension, uniform distribution, and a visible deposit and proved very adhesive. One mol. of strontium hydroxide, $\text{Sr}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, gave a neutral mixture at room temperature and a dried product similar to the barium precipitate in most respects. Eight-tenths mol. of calcium hydroxide, $\text{Ca}(\text{OH})_2$, as milk of lime, gave a neutral mixture at room temperature and completely precipitated the copper. The blue precipitate on drying, even in vacuo at 40° to 50°C ., was hard and gritty. Magnesium oxide, MgO , is obtained in two densities—light and heavy. Although slow to react, 1 mol. gave a neutral mixture and completely precipitated the copper at 40° to 60°C . and the resultant, while not bulky, was fairly soft.

With alkaline earth carbonates the general procedure was to dilute 7.49 gm. of copper sulphate with 50 to 150 c. c. of water and 0.8 mol. of the precipitant with 50 to 75 c. c., bring to temperature, add the copper to the carbonate, and continue the heating for the period specified, with thorough mixing, filter immediately, and dry slowly at moderate heat. Barium carbonate, BaCO_3 , is obtainable in two forms, the crystalline, or witherite, and the precipitated, a soft, bulky powder. The latter may be prepared from barium chloride and sodium carbonate, or from barium hydroxide and carbon dioxide. The crystalline powder failed to precipitate the copper, but 0.8 mol. of the precipitated carbonate gave a neutral mixture and completely removed the copper on long heating at 80° to 90°C . The light blue powder obtained was of poor texture and suspension and, although more conspicuous on foliage than the calcium carbonate precipitate, possessed relatively low fungicidal value, as determined by P. J. Anderson against the conidia of apple-scab fungus, *Venturia inaequalis* (Cke.) Wint. (Table 1.)

TABLE 1.—Effectiveness of basic sulphate as shown in preliminary experiments against conidia of apple-scab fungus

Strength applied	Number of slides	Average germination	Strength applied	Number of slides	Average germination
1 in 86.....	4	Per cent 3	1 in 171.....	2	Per cent 4
0 (control).....	4	49	0 (control).....	2	53

Strontium carbonate, SrCO_3 , is also obtainable in two forms, the crystalline and the precipitated. The crystalline, the only sample that was available, failed to precipitate the copper on long heating, even at 98°C . Magnesium carbonate is apparently available in three forms, the crystalline, $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$, and two basic carbonates, $4\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 5\text{H}_2\text{O}$ and $3\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 3\text{H}_2\text{O}$. Eight-tenths mol. of the first two gave neutral or slightly alkaline mixtures and completely precipitated the copper at a dilution of 150 c. c. in 30 minutes at 80° . The resulting blue precipitates were fairly soft and the greatest bulk was obtained with the monobasic tetracarbonate, giving a volume of about 4 c. c. per gram of copper sulphate employed.

Calcium carbonate, CaCO_3 , is obtainable in three forms, crystalline, chemically precipitated, and physically precipitated or triturated (whiting). The chemically precipitated, also known as precipitated chalk, may be prepared from calcium chloride and sodium carbonate, but in the United States at least is generally made from milk of lime and carbon dioxide and is divided into three grades according to density, i. e., light, medium, and heavy. The manufacturer of this product claims that the bulk of the several grades without tamping is approximately as shown in Table 2.

TABLE 2.—Density of calcium carbonate

Grade	1 cubic inch	1 cubic foot	1 pound	1 pound*
	Grams	Pounds	Cubic inches	Cubic inches
Light.....	5.00	19	92.0	83
Medium.....	6.33	24	72.0	58
Heavy.....	9.60	37	46.7	45

* Data by a chemical supply house.

The crystalline calcium carbonate failed to remove the copper in 120 minutes at 80°C ., but 0.8 mol. of the chemically precipitated gave a neutral mixture and completely precipitated the copper at various dilutions, periods of treatment, and temperatures. Little difference was noted between the several grades of the precipitated, but preference was given to the light as probably having greater surface area and more likely to assure complete reaction. The resulting blue precipitate was soft and bulky. While a number of the precipitants reported were sufficiently promising to warrant further study, calcium carbonate was selected for investigation both on account of the character of the resulting precipitate and because it is readily obtainable and comparatively inexpensive.

METHOD OF PREPARATION

In choosing the method of preparation, the character and volume of the precipitate, and the completeness of precipitation were the deciding factors, although time had also to be considered. In a measure color was a guide. A light or greenish-blue product proved of better quality and more bulky than a deeper blue. As 0.8 mol. of calcium carbonate per mol. of copper sulphate precipitated all the

copper, a greater quantity would simply reduce the concentration of basic sulphate and probably its fungicidal activity. A slight excess, 0.013 gm. per gram of copper sulphate, was generally added however to allow for moisture. The completeness of the reaction decreased with dilution and increased with the temperature and duration of treatment. With several variables more than one optimum might be expected.

At a dilution of 7.49 gm. of copper sulphate in 150 c. c. of solution, and 2.4 gm. of calcium carbonate in 150 c. c., and periods of treatment ranging from 10 to 105 minutes, with hand stirring, the relative volumes of fresh precipitate in the mother liquor after standing five minutes were as follows:

45° to 60° C.-----	Copper incompletely precipitated.
75°-----	34.3 (average of 12 tests).
80°-----	35.5 (average of 8 tests).
85°-----	37.7 (average of 9 tests).
95°-----	17.9 (average of 4 tests).

With 7.49 gm. of copper sulphate and 2.5 gm. of carbonate, and dilution, time, and temperature as stated, actual volumes in cubic centimeters of dry precipitate were obtained, as shown in Table 3.

TABLE 3.—*Volume of precipitate obtained with definite quantities of copper sulphate and calcium carbonate at different dilutions, temperatures, and periods of time*

Temperature	Duration of heating	Dilution 50 and 50 c. c.	Dilution 100 and 100 c. c.	Dilution 150 and 150 c. c.
° C.	Minutes	C. c.	C. c.	C. c.
50-----	100	28	35	Soluble copper.
60-----	55	61	47	Do.
65-----	60	71	57	Do.
70-----	60	67	62	Do.
75-----	60	78	48	63.
80-----	60	70	79	69.
85-----	60	63	70	71.
90-----	60	61	69	71.
98-----	60	50	53	57.

With the same weight of constituents, and with dilution, time, and temperature as stated, the bulks shown in Table 4 were obtained:

TABLE 4.—*Volume of precipitate obtained on heating definite quantities of copper sulphate and calcium carbonate at a constant dilution and temperature for different periods of time and filtering immediately and after standing 60 minutes*

Temperature	Duration of heating	Dilution	Filtered immediately	Filtered after standing 60 minutes
° C.	Minutes		C. c.	C. c.
80-----	15	150 and 50 c. c.-----	80	65
80-----	30	do-----	83	72
80-----	45	do-----	76	65
80-----	60	do-----	79	73

At a dilution of 100 and 50 c. c., 30 minutes, 80° C., and moderate stirring, with five different lots of precipitated calcium carbonate, the volumes of dry precipitate obtained were 86, 48, 65, 49, and 49 c. c., respectively. On repeating the tests with more thorough agitation the first sample of calcium carbonate gave dry precipitates of 79, 108, 103, 102, 71, 92, and 93 c. c., and the fifth sample of 97 and 65 c. c. At a dilution of 150 and 75 c. c., the first sample of carbonate gave 111 c. c. and the fifth 76 c. c. None of the preparations reported contained more than a trace of soluble copper by the ferrocyanide test, and in the last-mentioned cases none. As the reaction could not be controlled until the limiting factors were recognized, considerable variation was to be expected, but certain outstanding features were noted.

1. Substantially complete precipitation of the copper was obtained at 50° to 75° C. in high concentration.

2. With a total of 100 c. c. of water, the bulkiest precipitate was obtained at 75° C.; with 200 c. c. of water at 80°; and with 300 c. c. at 85°.

3. Immediate filtration at the completion of the reaction was preferable to allowing the mixture to stand.

4. The most voluminous precipitates were obtained in about 200 c. c. of water, in 30 minutes at 80° C. with thorough agitation, although other correlations may yield similar results.

Thorough stirring facilitated the reaction and increased the volume of the precipitate and, as a rule, the more bulky the basic sulphate the better is the suspension. By the adoption of an electrical stirrer more uniform results and a better product were obtained, and by regulating the flow of the dilute copper sulphate into the carbonate control was practically assured. The process finally evolved was as follows: 7.49 gm. of copper sulphate in 150 c. c. of solution heated to 80° C. were added in 5 to 6 minutes to 2.5 gm. of precipitated calcium carbonate, in 75 c. c. of water, at 80°, stirred vigorously for 30 minutes (including the period of precipitation) at 80°, filtered immediately, dried at a low temperature, and passed through a 0.5 mm. sieve. The yield of air-dry basic sulphate, calcium sulphate, and excess carbonate was 7.07 gm.—the average of 60 determinations.

TABLE 5.—*Volume of basic copper sulphate precipitate*

Grade	Number of samples	Precipitation period		Volume of precipitate		Volume of composite per gram, after re-sieving
		Range	Average	Range	Average	
		<i>Minutes</i>	<i>Minutes</i>	<i>C. c.</i>	<i>C. c.</i>	<i>C. c.</i>
1.-----	30	3.33-17.25	6+	97-115	105	15.63
2.-----	26	3.50-18.00	12+	72-94	81	12.35
3.-----	4	16.00-21.50	19+	66-70	68	-----

The volume of the basic sulphate varied inversely with the depth of color. Grade 1 (Table 5) was light blue, grade 2 a more decided blue, and grade 3 slightly darker. The color apparently increased with the amount of tetrabasic sulphate according to the hypothetical combination. (See Table 6.) The copper content of the crude

basic sulphate on an air-dry basis slightly exceeded that of crystalline copper sulphate (theoretical 25.457 per cent).

TABLE 6.—Composition of the three grades of basic copper sulphate

Components	Grade 1	Grade 2	Grade 3
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Water at 100° C., H ₂ O.....	7.880	8.900	9.280
Cupric oxide, CuO.....	32.732	32.356	32.356
Copper, metallic, Cu.....	(26.150)	(25.850)	(25.850)
Calcium oxide, CaO.....	17.750	17.770	17.880
Carbon dioxide, CO ₂803	.805	.870
Sulphur trioxide, SO ₃	30.930	30.790	-----
Unaccounted.....	9.905	9.379	-----
	100.000	100.000	-----
Hypothetical combination:			
Water, H ₂ O.....	7.038	7.521	-----
Tribasic sulphate, 4CuO.SO ₃	11.394	9.204	-----
Tetrabasic sulphate, 5CuO.SO ₃	28.383	30.033	-----
Calcium sulphate, CaSO ₄ .2H ₂ O.....	51.361	51.413	-----
Calcium carbonate, CaCO ₃	1.824	1.829	-----
	100.000	100.000	-----

Since the laboratory used by the writers is not equipped for quantity production, arrangements were made with a chemical firm to manufacture the basic copper sulphate. Fifty pounds were obtained for field work in 1925 and 250 pounds in 1926. The 1925 product was distinctly blue, flourlike, and of poor flow. The first shipment of 1926 was lighter blue and of better flow, but the second was less satisfactory. The density and copper content of the commercial product are given in Table 7.

TABLE 7.—Density and copper content of the commercial basic copper sulphate

Shipments	Volume per gram after resieving	Copper content
	<i>C.c.</i>	<i>Per cent</i>
1925.....	5.94	26.38 (9.12 H ₂ O)
1926, first.....	7.94	26.50 (8.80 H ₂ O)
1926, second.....	6.21	26.31 (8.11 H ₂ O)

LABORATORY TESTS OF FUNGICIDAL EFFICIENCY

The activity of basic copper sulphate against the conidia of apple-scab fungus, *Venturia inaequalis* (Cke.) Wint., was determined by P. J. Anderson, formerly of the department of botany of this station. Glass slides were sprayed on one end with the mixture at the strength stated, the other end serving as a control, and exposed to the air for 24 hours. Drops of water containing spores of the fungus were then placed on the slides, which were kept in a moist chamber, and the percentage of germination was determined at the end of 24 or 48 hours (substantially the method of Reddick and Wallace (14). See Table 8.

TABLE 8.—Effectiveness of basic sulphate against conidia of apple-scab fungus

Copper in the fungicide	Strength of application	Copper content of spray	Number of slides	Germination average
	Grams	Per cent		Per cent
26.50 per cent.....	1 in 150 c. c.....	0.176	4	0 in 24 hours. ^a
	0 (control).....		4	47 in 24 hours.
26.50 per cent.....	1 in 300 c. c.....	.088	2	0 in 24 hours. ^a
	0 (control).....		2	30 in 24 hours.
26.50 per cent.....	1 in 150 c. c.....	.176	2	0 in 48 hours.
	0 (control).....		2	20 in 48 hours.
26.50 per cent.....	1 in 300 c. c.....	.088	2	0 in 48 hours.
	0 (control).....		2	20 in 48 hours.
26.50 per cent.....	1 in 600 c. c.....	.044	2	Trace in 48 hours. ^b
	0 (control).....		2	20 in 48 hours.

The last three entries are repeated tests.

^a Unchanged after 72 hours.

^b The term "trace" indicates that a few spores produced abnormal short germ tubes.

Samples of the three grades of basic sulphate were submitted to William L. Doran, of the department of botany, for additional tests with spores. He employed spores of the following fungi, using the method of Reddick and Wallace (14): Apple scab, *Venturia inaequalis* (Cke.) Wint.; carrot leaf spot, *Cercospora apii* Fres. var. *carotae* Pass.; gray mold of lettuce, *Botrytis cinerea* Pers.; carrot blight, *Macrosporium carotae* E. and L.; snapdragon rust, *Puccinia antirrhini* Diet. and Holw.

As a basis of comparison, Bordeaux mixture of different concentrations was used, as follows:

Bordeaux mixture	Approximate copper content (per cent)
8.00 : 8.00 : 50.....	0.5000
4.00 : 4.00 : 50.....	.2500
2.00 : 2.00 : 50.....	.1250
1.00 : 1.00 : 50.....	.0625
.50 : .50 : 50.....	.0312
.25 : .25 : 50.....	.0156
.125 : .125 : 50.....	.0078

TABLE 9.—Inhibiting effect of copper in Bordeaux mixture and in basic copper sulphate

Fungi	Per cent of copper in fungicide as used				Effect on germination
	Bordeaux mixture 1:1	Basic copper sulphate No.			
		1	2	3	
V. inaequalis.....	0.0078	0.0078	0.0078	0.0078	Germination.
	.0156	.0156	.0156	.0156	No germination.
C. apii var. carotae.....	.0312	.0625	.0625	.0625	Germination.
	.0625	.1250	.1250	.1250	No germination.
B. cinerea.....	.0156	.0156	.0156	.0156	Germination.
	.0312	.0312	.0312	.0312	No germination.
M. carotae.....	.0625	.1250	.1250	.1250	Germination.
	.1250	.2500	.2500	.2500	No germination.
P. antirrhini.....	.5000	.5000	.5000	.5000	Germination.
					No germination.

The three grades of basic copper sulphate (Table 9) proved equally effective, but less so than Bordeaux mixture with the more resistant fungi.

FIELD TESTS

Laboratory tests, such as reported, are undoubtedly indicative of relative toxicity, but since control in the field is the main objective experiments were conducted by E. F. Guba at the Market Garden Field Station at Waltham, Mass., during the season of 1925 on cucumbers and celery. The cucumbers were inoculated with anthracnose, *Colletotrichum lagenarium* (Pass.) Ells. and Hals., on July 21, about two and one-half weeks after vining had begun (July 3), and with downy mildew, *Peronoplasmopara cubensis* (Berk. and Curt.) Rostew., on July 30. The former disease appeared in "noticeable proportions" in about 10 days and spread rapidly in the control plot, but the latter failed to develop. The picking season extended from July 24 to September 2.

The applications of spray or dust were made on July 3, 9, 20, 30, and August 7, 15, and 20. The results obtained in control of the disease on cucumbers are shown in Table 10.

TABLE 10.—Relative effectiveness of three fungicides used for the control of anthracnose of cucumbers

Fungicide	Copper content of spray or dust	Number of applications	Number of rows treated	Yield of cucumbers		Gain over control	
				Number	Pounds	Number	Pounds
Bordeaux mixture (4:4:50)-----	<i>Per cent</i> 0.25	7	3	<i>Number</i> 1,193	<i>Pounds</i> 915	<i>Per cent</i> 20.63	<i>Per cent</i> 26.91
Basic copper sulphate-----	.25	7	3	1,329	1,055	34.38	46.32
Copper lime dust (proprietary)-----	5.00	7	1	385	293	16.78	21.91
Control-----	0	0	3	989	721	-----	-----

Observations during the growing season, together with the yield obtained, indicate that basic copper sulphate gave as satisfactory control of anthracnose as Bordeaux mixture.

In the celery experiment, the variety Golden Plume was employed. This variety is very susceptible to the causal organisms of both the early and late blights, *Cercospora apii* Fr. and *Septoria apii* Chester. The former disease appeared naturally, and the latter was introduced by inoculation on July 20. The celery was boarded on August 31 and harvested September 10. The spraying was done on June 15 and 26, July 1, 15, and 30, and August 7, 15, 20, and 28. The results obtained are shown in Table 11.

TABLE 11.—Relative effectiveness of Bordeaux mixture and basic copper sulphate used for the control of early and late blights of celery

Fungicide	Copper content of spray	Number of applications	Yield			Gain over control		
			Number of crates	Total weight	Average weight	Number of crates	Total weight	Average weight
Bordeaux mixture (4:4:50)-----	<i>Per cent</i> 0.25	9	14.66	<i>Pounds</i> 484.0	<i>Pounds</i> 33.0	<i>Per cent</i> 25.73	<i>Per cent</i> 73.48	<i>Per cent</i> 38.08
Basic copper sulphate-----	.25	9	14.60	473.5	32.4	25.21	69.71	35.56
Control-----	0	0	11.66	279.0	23.9	-----	-----	-----

* The crates contained 18 bunches with 3 stalks to the bunch.

Observations and yield indicate approximately equal control. The crop on the check plot was greatly reduced by late blight and had no first-grade product.

The adhesiveness of basic copper sulphate is naturally inferior to that of freshly prepared Bordeaux mixture, which is of a gelatinous character. While this deficiency does not appear to constitute a serious objection, it was deemed advisable to add adhesives and spreaders to ascertain whether their influence was appreciable. With cucumbers, 4 gm. of glue (well dispersed) were incorporated with each gallon of spray on July 3 and 20, 1 ounce of rosin fish-oil soap on July 9 and 1.8 gm. of dextrin on July 30. With celery, 4 gm. of glue were added on June 26 and July 1, and 4.5 gm. on August 28; 0.5 ounce of rosin fish-oil soap on July 15, and 1.8 gm. of dextrin on July 30. On the remaining dates, with both cucumbers and celery, no adhesive was added. Further trials on a small scale were made on cucumbers, celery, and tomatoes with from 2 to 5.7 gm. of dextrin, albumin, gelatin, gum arabic, and glue, of which the two latter and rosin fish-oil soap appeared the more promising from a visual inspection of the residue after washing. Soap is objectionable in that it forms a curdy precipitate. Guba also employed 4.5 gm. of calcium caseinate to the gallon of spray, but he did not note any appreciable improvement except possibly in spreading. The work is being continued with a variety of compounds and the actual quantity of copper on a given area of leaf surface determined before and after washing.

SUMMARY

The crude basic copper sulphate described is a light, bulky powder, of good flow, free from grit, and adapted for application as a spray or dust.

The copper content is similar to that of copper sulphate and can presumably be applied at the same concentration as Bordeaux mixture.

Basic copper sulphate is an effective fungicide, easily applied, fairly indicative of the leaf surface covered, and of reasonable suspension, distribution, and adhesiveness.

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THE LINKAGE OF PUBESCENT NODE AND BEARD FACTORS AS EVIDENCED BY A CROSS BETWEEN TWO VARIETIES OF WHEAT¹

By E. F. GAINES, *Cerealist, Washington Agricultural Experiment Station, and*
ARTHUR CARSTENS, *graduate student, Washington State College*

Linkage groups in wheat have not formerly been definitely established, but the presence of two bearded, smooth-noded plants found in the F_2 generation of a cross of Hybrid 128 \times Velvet Node grown in 1924 made it seem likely that crossing over occurred within the bearded-pubescent node linkage group. This supposition was strengthened by the fact that Love and Craig² had found aberrant cases in a cross between Velvet Node and "New Columbia" which were not at first definitely known to be crossovers, but which subsequent testing has probably proved to be such.

Hybrid 128, Washington No. 592 and Cereal Investigations No. 4512, has an awnless, club head, white seed, and stiff straw. This winter wheat was developed at the Washington Agricultural Experiment Station, and is commonly grown in eastern Washington.

The Velvet Node parent is the offspring of a single plant found in a field of spring wheat near Colton, Wash., in August, 1919, and has been Washington No. 1981. It is a bearded spring variety, with long head and red grain. In addition, it has a hairy node like that of Cereal Investigations accession No. 5877.

The seeds obtained from crossing these two varieties were sown in the fall of 1922, and three F_1 generation plants were harvested the next summer. These plants were all beardless, having club heads, pubescent nodes, red grain, and spring habit of growth.

Part of the seeds produced in this F_1 generation were rolled in smut and part were treated with copper carbonate dust before planting in the fall of 1923. Of the 393 smut-free plants harvested from the treated row, 189 were breadless and possessed pubescent nodes, 112 were bearded and possessed pubescent nodes, 90 were beardless and possessed a glabrous node, and 2 were bearded and possessed a glabrous node.

A 9 : 3 : 3 : 1 ratio had been expected, but with the exception of the two bearded plants with glabrous node the results approached a 2 : 1 : 1 ratio. This indicated a very close if not complete linkage of the factors producing beards and pubescent nodes. The presence of the two aberrant cases was the only evidence in the F_2 generation that crossing over had occurred.

In the spring of 1925 these F_2 generation seeds were sown. Three hundred and eighty-three rows of plants were produced from which the gametic composition of the F_2 generation could be interpreted. The results are shown in Table 1. The factor for beardlessness is denoted by B , for the bearded condition by b , for pubescent node by V , and for glabrous node by v .

¹ Received for publication May 20, 1926; issued October, 1926. Published with the approval of the director of the Washington Agricultural Experiment Station as Scientific Paper No. 132, College of Agriculture and Experiment Station.

² LOVE, H. H., and CRAIG, W. T. THE INHERITANCE OF PUBESCENT NODES IN A CROSS BETWEEN TWO VARIETIES OF WHEAT. Jour. Agr. Research 28: 841-844. 1924.

TABLE 1.—The gametic composition of the F_2 generation as indicated by the segregation in the F_3 generation, compared with the theoretical composition based on the total number of crossover gametes actually occurring

Genotypes	Actual	Theoretical	Difference
Homozygous:			
<i>BBVv</i> (crossover) ^a	0	0.3	-0.3
<i>Bbvv</i> (noncrossover).....	78	86.0	-8.0
<i>bbVv</i> (noncrossover).....	95	86.0	+9.0
<i>bbvv</i> (crossover) ^a	2	.3	+1.7
Heterozygous:			
<i>BbVv</i> (crossover) ^a	0	.5	-.5
<i>Bbvv</i> (noncrossover).....	172	172.0	0
Mixed:			
<i>BbVv</i> (crossover).....	6	9.5	-3.5
<i>BbVv</i> (crossover).....	7	9.5	-2.5
<i>Bbvv</i> (crossover).....	8	9.5	-1.5
<i>bbVv</i> (crossover).....	15	9.5	+5.5
Total.....	383	383.1	-.1

^a Crossing over in both gametes; each of the other four crossovers contains one crossover and one non-crossover gamete.

The calculation of the theoretical numbers according to Jennings's formulae³ is briefly explained in the following paragraphs.

The 383 plants represented 766 gametes, of which 726 showed the same combination of factors as occurred in the foregoing generation of gametes and 40 showed other combinations. The 36 cases in which crossing over occurred in one of the gametes represented 36 and the 2 cases in which crossing over occurred in both represented 4 of the 40 gametes last mentioned. The linkage ratio, r , is therefore calculated as follows: $\frac{726}{40} = 18.15$.

The formulae as applied to this problem are copied below. The letters B , b , V , and v are substituted for A , a , B , and b of Jennings's formulae, and the case in which the original parents, i. e., the F_1 generation, are $BbvV$, is taken instead of $BbVv$ used by Jennings. In other words, a dominant and recessive character are linked, instead of two dominants. Linkage equals r in both sets of gametes. The results give the zygotic constitution of a theoretical population of one by any number of successive self-fertilizations. Since n equals 1 in this problem, the formulae become quite simple and are conveniently applied without the use of the recurring common surds v and w . These specifically applied formulae are given in next to the last column of Table 2. The last column gives the numerical results of substituting 18.15 as the value of r . These figures multiplied by the 383 zygotes considered give the theoretical numbers shown in Table 1.

The percentage of crossover, however, conveys a more concise and comparable impression of the degree of linkage than any other single figure. It is also the basis for the construction of chromosome maps.⁴

The percentage of crossover is calculated according to the conceptions obtained from Sinnott and Dunn⁵ as follows: $\frac{40}{766} + 100 = 5.222$.

³ JENNINGS, H. S. THE NUMERICAL RESULTS OF DIVERSE SYSTEMS OF BREEDING, WITH RESPECT TO TWO PAIRS OF CHARACTERS, LINKED OR INDEPENDENT, WITH SPECIAL RELATION TO THE EFFECTS OF LINKAGE. *Genetics* 2: 154. 1917.

⁴ MORGAN, T. H. THE THEORY OF THE GENE. p. 24, illus. New Haven and London. 1926.

⁵ SINNOTT, E. W., and DUNN, L. C. PRINCIPLES OF GENETICS. AN ELEMENTARY TEXT, WITH PROBLEMS. p. 160, 161, 168. New York and London. 1925.

To show the relationship between linkage ratio denoted by r and percentage crossover denoted by p , the following formulae were derived:

$$p = \frac{100}{r+1}$$

$$r = \frac{100-p}{p}$$

TABLE 2.—*Formulae for the zygotic constitution of the population derived from the P_1 parent $BbVv$ by any number of successive self-fertilizations; also formulae adapted to the F_2 generation and results obtained when $r=18.15$*

Let r = the linkage ratio
 n = the number of successive self-fertilizations

$$v = \frac{r^2+1}{2(r+1)^2}$$

$$v^n = \frac{r^{2n}+1}{2(r+1)^2}$$

Genotypes	After n generations	If $n=1$	If $n=1$ and $r=18.15$
$BBVV$	$\frac{2^n-1}{2^{n+1}} + \frac{v^n-w^n-w^{n-1}-w^{n-2}-\dots-w}{4}$	$\frac{1}{4(r+1)^2}$	0.0007
$BbVv$	$\frac{2^n-1}{2^{n+1}} + \frac{v^n+w^n+w^{n-1}+w^{n-2}+\dots+w}{4}$	$\frac{r^2}{4(r+1)^2}$.2246
$bbVV$	Same as $BbVv$	Same as $BbVv$2246
$bbvv$	Same as $BBVV$	Same as $BBVV$0007
Total homozygotes.....	$\frac{2^n-1}{2^{n-1}} + v^n$	$\frac{r^2+1}{2(r+1)^2}$.4505
$BbVv$	$\frac{v^n-w^n}{2}$	$\frac{1}{2(r+1)^2}$.0014
$Bbvv$	$\frac{v^n+w^n}{2}$	$\frac{r^2}{2(r+1)^2}$.4491
Total heterozygotes.....	v^n	$\frac{r^2+1}{2(r+1)^2}$.4505
$BBVv$	$\frac{1}{2^{n+1}} - \frac{v^n}{2}$	$\frac{r}{2(r+1)^2}$.0247
$BbVV$	Same as $BBVv$	Same as $BBVv$0247
$Bbvv$	Same as $BBVv$	Same as $BBVv$0247
$bbVv$	Same as $BBVv$	Same as $BBVv$0247
Total mixed.....	$\frac{1}{2^{n-1}} - 2v^n$	$\frac{2r}{(r+1)^2}$.0990
Total population.....	1.....	1	1.0000

The actual counts correspond to the theoretical expectation closely enough to warrant the conclusion that linkage exists between the beard and pubescent node factors and that the degree of linkage is such that about 5 per cent of crossing over occurs. It will be noted that the bearded groups in every case were larger than expected, and this prolificacy in regard to beards was proportionately greater in the crossover classes.

AN APPARENTLY NEW SUGAR-CANE MEALYBUG¹

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INTRODUCTION

The mealybug described below has been reported as injurious to sugar cane in certain localities in Cuba, and is now the subject of study by the entomologists of the Tropical Plant Research Foundation. A review of the systematic literature on the group to which it has been assigned indicates that the species has not yet been described, and this description is therefore offered in order to make it known and to facilitate reference to it in any future publications regarding its injuriousness.

GENUS RIPERSIA SIGNORET

The genus *Ripersia*, as now accepted, does not contain a well defined, homogeneous group of species, but instead is little more than a dumping ground for species of mealybugs having 6-segmented antennae and living on members of the Gramineae, or on the roots of other plants or in ants' nests. Such being the case, few precise suggestions can be offered respecting the actual relationship of the form now described.

RIPIERSIA RADICICOLA, new species

Described from alcoholic specimens only; no information available regarding the character and extent of the external secretory covering.

Occurs on the roots of the host plants.

Adult female.—Very stout oval, almost circular in outline, strongly convex. length of fully developed form as mounted 3.5 mm., width about 3.25 mm.; antennae 6-segmented, placed rather close together, although not contiguous, lengths of segments of one in microns as follows: I, 53; II, 50; III, 70; IV, 28; V, 43; VI, 86; segment V with one, VI with three to four stout setae in addition to slender setae; legs short and small but stout, not otherwise unusual; measurements of a posterior leg in microns as follows: Coxa, 200; trochanter, 90; femur, 168; tibia, 136; tarsus, 90; claw, 34; hind coxa with numerous, but scattered, tiny pores on both faces, claw without denticle, claw digitules threadlike but distinctly knobbed at apices, barely reaching tip of claw, tarsal digitules more slender, not knobbed; beak elongate conical, distinctly 2-segmented, length about 195 μ ; both anterior and posterior pairs of ostioles present, small and inconspicuous; only the posterior apical pair of cerarii developed, each made up of a pair of small conical spines and a loose cluster of triangular pores; anal lobes, as such, wholly undeveloped, apical seta placed

¹ Received for publication May 7, 1926; issued October, 1926.

immediately beneath apical cerarius, without chitinized thickening adjacent, each seta about $200\ \mu$ long; body with triangular pores, scattered fairly uniformly, more numerous along the margin and posteriorly, with relatively few multilocular disk pores around the vaginal opening, and with numerous large tubular ducts in loose clusters along the abdominal margin, a few scattered along the margin anteriorly, and in transverse rows ventrally in the abdominal region; body setae few, small, slender, inconspicuous, most abundant adjacent to the duct clusters, anal ring slender with two rows of pores and six setae, the longest of these about $118\ \mu$; ventral cicatrix not located, apparently not present.

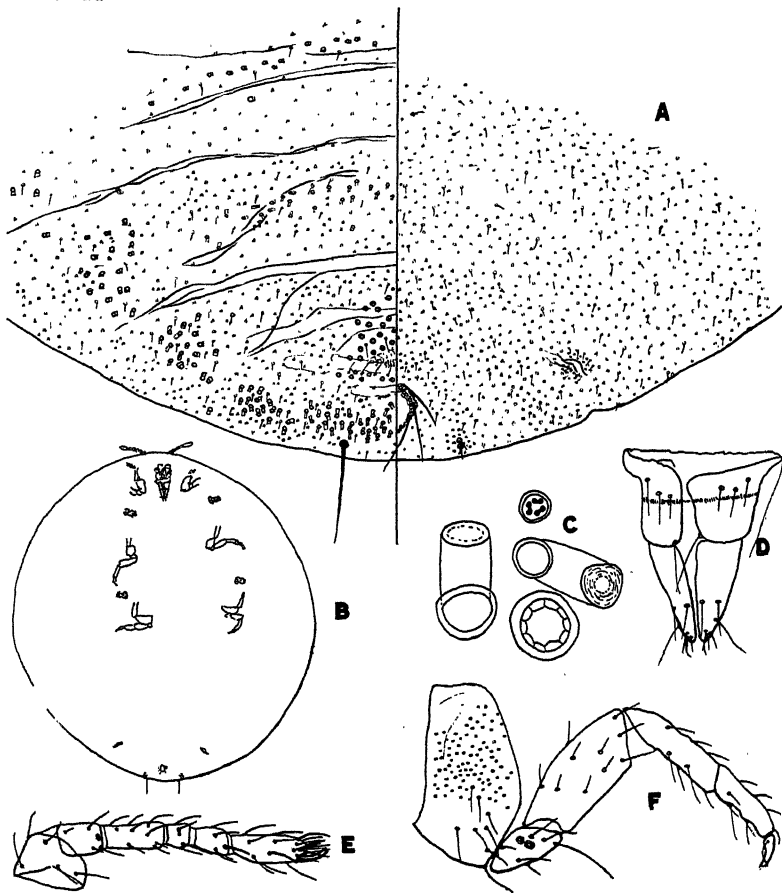


Fig. 1.—*Ripersia radicolica*, adult female. A, apex of abdomen, dorsal and ventral, $\times 230$; B, outline of body, optical section, $\times 12$; C, types of body pores, $\times 1275$; D, beak, $\times 120$; E, antenna, $\times 120$; F, leg, $\times 120$.

This species has been described after an examination of microscopic slide mounts from the following lots of material: On roots of sugar cane, Jobabo, Cuba, January 21, 1925, collector C. F. Stahl (Tropical Plant Research Foundation No. 14a Ent.) (holotype and paratypes); the same, December 3, 1924 (T. P. R. F. No. 14 Ent.) (paratypes); the same, collected January 23, 1925, T. P. R. F. No. 14b Ent.) (paratypes); on Crystallina roots, same locality, forwarded by W. A. Orton, August, 1924 (paratypes); Central Baragua, Cuba,

on sugar cane, May 6, 1925 (T. P. R. F. No. 1081 Ent.) (paratypes), and May 13, (T. P. R. F. No. 1097 Ent.) (paratypes); same locality on grasses; May 6 (T. P. R. F. No. 1082 Ent.) (paratypes); on cane roots, eastern Cuba, collector S. C. Bruner (No. 9027), received with letter of December 14, 1923 (paratypes); Soledad, Cuba, February 27, 1925, collector J. G. Myers (No. 591) (paratypes); Habanilla, Cuba, April 7, 1925, collector J. G. Myers (No. 684) (paratypes); on roots of *Echinochloa colonum*, Baragua, Camaguey, Cuba, April 21, 1926, collected by C. F. Stahl (paratypes).

The types of the species are in the United States National Collection of Coccidae.

The insect herein described has been compared with specimens or descriptions of all of the mealybugs that have been reported to occur on sugar cane. It has also been compared with all of the species of *Ripersia* represented in the national collection. Of these it resembles the species *Ripersia anomala* Newst. and *R. aurantia* Cockerell in that all have clusters of relatively large, short tubular ducts present along the body margin; it differs from both of these in several particulars, but most conspicuously in having only the single posterior apical pair of cerarii developed, the other two species having the series of marginal cerarii complete, or practically so.

No definite statement can be made as to the original home of the species. The very limited information now available suggests the possibility that it is native to Cuba and has transferred its activities from some indigenous plant, probably a grass, to sugar cane.

A CONTRIBUTION TO THE BACTERIOLOGICAL STUDY OF HAM SOURING¹

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INTRODUCTION

"Ham souring" is a term applied in the meat-packing industry to any sour, unsound, or putrid condition developing in the interior of the ham. It may vary in degree from a slight taint in a limited area to a putrid condition affecting almost the entire ham.

The first systematic scientific study of ham souring was conducted by McBryde,² who concluded from his investigation that ham souring is caused by an anaerobic bacillus to which he gave the name *Bacillus putrefaciens*. He reported the isolation of this bacillus from sour hams, its cultivation in the laboratory, the production of typical souring by inoculating sound hams with the cultures, and recovery of the bacillus from the hams soured by this means. McBryde assumed that this bacillus is present in the dust and dirt of packing houses and gains entrance to the hams through the various manipulations to which they are subjected.

In a previous communication³ the writer showed that the interior portions of fresh, chilled hams are never sterile, but harbor numerous species of bacteria, both aerobic and anaerobic, including *Bacillus putrefaciens* and other bacteria of the type commonly associated with ham souring. The presence of a similar flora in the lungs of hogs slaughtered in accordance with regular practice was also reported. The conclusion drawn was that every ham must be regarded as a potential sour ham containing the organisms which would cause spoilage if permitted to develop.

During the interval since the appearance of the publication last cited, A. F. Reith, working under the Arthur Lowenstein fellowship placed at the University of Chicago through the Institute of American Meat Packers, has been engaged in an investigation of the subject. Through the courtesy of J. F. Norton, directing the fellowship, and W. Lee Lewis, director of the Bureau of Scientific Research in the Institute of American Meat Packers, an opportunity was afforded in March, 1925, for an exchange of views on Reith's findings and the data obtained by the writer as here set forth.

THE PROBLEM

The finding of various species of bacteria in the interior of hams, including the bone marrow, 48 hours after slaughter, together with the fact that during such time the carcasses had been held at a low

¹ Received for publication June 5, 1926; issued October, 1926.

² MCBRYDE, C. N. A BACTERIOLOGICAL STUDY OF HAM SOURING. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 132, 55 p., illus. 1911.

³ BOYER, E. A. A STUDY OF THE SPOILAGE OF HAMS AND OTHER PORK PRODUCTS. Amer. Food Jour. 18: 197-200. 1923.

temperature in the chill room, made it appear probable that such organisms were present at the time the carcasses were removed from the killing floor. This supposition having been confirmed, as shown by data presented later, further investigation was directed toward locating the source of the organisms.

Three possible sources were suggested—first, that the organisms are present in the blood and tissues of the living animal before slaughter; second, that they are rapidly disseminated throughout the carcass after death; and, third, that they gain access during the various manipulations to which the carcass is subjected on the killing floor. The last-mentioned hypothesis appearing most probable, attention was first given to it.

METHODS AND PLAN OF WORK

The methods used and the plan of work were such as would serve the purpose in hand rather than those suited to an exhaustive investigation of the subject.

The hams used were obtained from a local establishment operating under Federal inspection. Not over one hour elapsed from the time the hams were cut from the carcasses until the cultures were removed. Cultures were taken from each ham from one or more of the following locations: Flesh in close proximity to the stifle joint; bone marrow of the femur; and synovial fluid of the stifle joint. All instruments, media, and solutions used were sterilized in accordance with the usual methods. Care was taken throughout to prevent extraneous contamination.

The hams were sectioned with a heavy knife heated to such temperature as thoroughly to sear the cut surfaces. The area from which cultures were to be taken was again seared on the exposed surface, and portions of the flesh were taken from beneath this area by means of scalpel and forceps. In taking cultures of the joint fluid the meat was carefully removed, leaving intact the femoropatellar capsule. The exposed surface was then seared and a small incision made through which the fluid was removed with a platinum loop. Cultures of the bone marrow were taken close to the nutrient foramen, at which point the marrow usually shows a collection of blood.

ANAEROBIC GROWTH

Portions of approximately 3 gm. each of the flesh and bone marrow were planted into separate tubes of cooked meat medium and incubated for eight days. Incubation was conducted for two days at 37.5° C. and at room temperature for the remainder of the period. From those tubes showing evidence of anaerobic growth, such as digestion of the medium, gas formation, or foul odor, stained smears were examined microscopically. For isolation the tubes were heated to 80° to 85° C. to eliminate vegetative forms, and deep shake cultures were made in glucose agar, the treatment being repeated until an evidently pure culture was obtained. It is, of course, recognized that by such procedure some specimens may have been regarded as free from anaerobes which would have been found otherwise by more intensive examination.

AEROBIC COUNT

A 3 to 5 gm. portion of the flesh was disintegrated as much as possible by shaking for approximately one minute with ground glass in 10 c. c. of sterile distilled water. No decided disintegration of the meat could be obtained in this manner. A 1 c. c. portion was then planted on plain nutrient agar and incubated for 48 hours at 37.5° C. The aerobic count in the data given represents, therefore, the number of organisms obtained from 0.3 to 0.5 gm. of flesh. In the case of the joint fluid two loopfuls were used for each plate and the aerobic counts given represent the number of colonies developed from that quantity.

BACILLUS COLI

Portions of the flesh and bone marrow similar in quantity to those used for anaerobic bacteria and portions of the joint fluid such as were used for aerobic count were planted direct into lactose bile. In addition, 1 c. c. quantities of the preparation of the flesh used for aerobic plates were planted into lactose bile. From all tubes giving presumptive evidence of *B. coli* through gas formation in the lactose bile, streak cultures were made on Endo's medium for confirmation.

TESTS ON HAMS TAKEN FROM KILLING FLOOR

Six hams, each from a different carcass, were removed as soon as killing-floor operations had been completed and before the carcasses were run into the cooler. Under conditions prevailing at the establishment at the time these specimens were taken, approximately 30 minutes elapsed between the sticking of the animals and evisceration, and about 15 minutes more before the samples were taken. The results obtained on examination of these six hams in accordance with the methods detailed above are shown in Table 1.

TABLE 1.—Results of examination of hams from hog carcasses 45 minutes after slaughter

Specimen	Anaerobes	Aerobic colonies	<i>B. coli</i>
No. 112:			
Flesh.....	Found.....	9	Not found
Joint fluid.....	(a).....	5	Do.
Bone marrow.....	Found.....	(a)	Do.
No. 113:			
Flesh.....	Not found.....	30	Do.
Joint fluid.....	(a).....	20	Do.
Bone marrow.....	Found.....	(a)	Do.
No. 114:			
Flesh.....	Not found.....	21	Do.
Joint fluid.....	(a).....	4	Do.
Bone marrow.....	Found.....	(a)	Do.
No. 115:			
Flesh.....	Not found.....	78	Do.
Joint fluid.....	(a).....	32	Do.
Bone marrow.....	Not found.....	(a)	Do.
No. 116:			
Flesh.....	Found.....	9	Do.
Joint fluid.....	(a).....	6	Do.
Bone marrow.....	Found.....	(a)	Do.
No. 117:			
Flesh.....	do.....	14	Do.
Joint fluid.....	(a).....	3	Do.
Bone marrow.....	Not found.....	(a)	Do.

* No test.

This evidence of the existence of microorganisms in the interior of the hams 45 minutes after slaughter is in accord with the results obtained in the former work from examination of 29 hams taken at the conclusion of the chilling period, and shows that the organisms found were present at the beginning of the chilling period.

A number of species of anaerobes were represented in the cultures obtained, and reference to their presumptive identification will be made below. There were isolated a numerous and varied group of aerobic organisms: Coccus and rod forms, motile and nonmotile; gelatin liquefiers and nonliquefiers, chromogenic and nonchromogenic.

The absence of the *Bacillus coli* group of organisms from the numerous cultures taken from these hams is of particular interest. The members of this group are abundant and ubiquitous on the killing floor, and are almost invariably found on the surfaces of the carcasses which are exposed during killing-floor operations. Their absence is of special significance in that it goes far to eliminate the possibility that the organisms present in the hams gain access during killing-floor operations.

Because of the temperatures prevailing, the scalding and dehairing operations are exceptions. Examination of several samples of the water used in these processes showed that the temperature prevailing (140° to 145° F.) eliminates *Bacillus coli*, while the spore-bearing anaerobes and other heat-resistant organisms are abundant. The bacterial flora of the scalding vat, like that of the lungs, bears a striking similarity, in its general character at least, to that of the carcass. The possibility of the dirty vat water entering through the stick wound into the circulatory channels and, abetted by the beaters of the dehairing machine, being rapidly and widely distributed throughout the carcass appeared to warrant consideration.

In order to obtain information on this point, the stick wounds of four carcasses were securely ligatured so as to preclude the possibility of contamination entering in that manner during the scalding and dehairing. A ham was taken from each of the dressed carcasses and examined, with the results shown in Table 2.

TABLE 2.—Results of examination of hams from carcasses with stick wounds ligatured during scalding and dehairing

Specimen	Anaerobes	Aerobic colonies	B. coli
No. 136:			
Flesh.....	Not found.....	73	Not found
Joint fluid.....	(a).....	40	Do.
Bone marrow.....	Not found.....	(a)	Do.
No. 137:			
Flesh.....	do.....	25	Do.
Joint fluid.....	(a).....	6	Do.
Bone marrow.....	Found.....	(a)	Do.
No. 138:			
Flesh.....	do.....	38	Do.
Joint fluid.....	(a).....	6	Do.
Bone marrow.....	Found.....	(a)	Do.
No. 139:			
Flesh.....	do.....	7	Do.
Joint fluid.....	(a).....	0	Do.
Bone marrow.....	Not found.....	(a)	Do.

* No test.

As a further test, two carcasses were taken direct from the bleeding rail, scalded in a steam cooker, and the hair removed by hand scraping. Examination of the hams from these carcasses gave results consistent with those previously obtained.

Finally, the hams removed from four carcasses taken directly from the bleeding rail and neither scalded nor dehaired, were examined. The hams were removed from these carcasses as soon as bleeding had been satisfactorily completed and cultures were taken immediately from the flesh and bone marrow (Table 3).

TABLE 3.—Results of examinations of hams from carcasses direct from bleeding rail

Specimen	Number of cultures taken	Number positive	Number showing anaerobes
No. 142:			
Flesh.....	2	2	0
Bone marrow.....	1	1	1
No. 143:			
Flesh.....	2	2	1
Bone marrow.....	1	1	1
No. 144:			
Flesh.....	1	0	0
Bone marrow.....	1	1	0
No. 145:			
Flesh.....	2	1	1
Bone marrow.....	1	0	0

The results of this investigation showed that the bacteria present in the chilled carcass do not gain access to it through any of the operations on the killing floor, but are present immediately after the death of the animal and before the carcass has been subjected to any of the killing-floor operations. No attempt was made to determine whether the bacteria found were the result of rapid, agonal invasion or whether they are present in the blood and tissues of the living animal. While this question is one of scientific interest, it was not within the scope of the present investigation.

CHARACTERISTICS OF THE BACTERIA FOUND

The demonstration of anaerobic bacteria in the hams taken directly from the carcasses on the bleeding rail is of particular interest and importance, since it is organisms of this type which are responsible for the souring of ham.

From anaerobic cultures of the hams taken from the killing floor there were isolated five distinct species. Four of these have been tentatively identified as *Bacillus putrefaciens*, *B. histolyticus*, *B. sporogenes*, and *B. tertius*. The fifth species has not been identified.

No thought is entertained that all the species of anaerobes occurring in fresh hams were isolated. For instance, the unidentified organism was overlooked for a long period. When it was finally isolated and familiarity with its morphological characteristics attained, it was recognized as an organism which had been observed repeatedly in the smears from the anaerobic cultures. Further, there was obtained toward the close of the work a species which, although culturally resembling *Bacillus sporogenes* and tentatively classed as such, presented a unique morphology which made the tentative identification

questionable. Other characteristics or peculiarities are noted in the comments on each particular organism.

BACILLUS PUTREFACIENS

B. putrefaciens was found frequently in fresh hams and has been repeatedly isolated from sour hams. It was the only organism isolated which formed perfectly round terminal spores. Fully developed spores were formed in deep colonies in glucose agar after seven days' incubation. When grown in pure culture in the cooked meat medium used, this organism did not form spores. In mixed cultures from hams, on the contrary, it was often found to form spores freely after five to seven days' incubation in this medium. This organism is primarily of the saccharolytic group and gives no evidence of digestion of the solid particles in cooked meat medium. Its optimum temperature for growth was from 20 to 25° C. In shake cultures in glucose agar incubated at 37.5° there occurred in the lower part of the medium a marked haziness, but colonies of visible size did not develop at this temperature.

BACILLUS HISTOLYTICUS

B. histolyticus has been repeatedly isolated from fresh hams and has often been isolated from hams and shoulders which were found to be spoiled at the end of 48 hours in the chill room. In some such cases the odor of the affected areas was characteristic of that produced in vitro by this bacillus. This organism is distinguished from all of the other anaerobes herein considered by the fact that it does not produce any gas bubbles when grown in deep colonies in glucose agar and no fragmentation of that medium even after four days' incubation. This is in marked contrast to the action of *B. sporogenes* which otherwise is somewhat similar in its growth, particularly in the cooked meat medium. The extremely rapid proteolytic action of this organism in the meat medium and early deposition of white crystals were marked and characteristic features.

BACILLUS SPOROGENES

B. sporogenes was distinguished by the fact that it showed greater tolerance of oxygen than any of the other anaerobic forms studied. In glucose agar the colonies would extend to within 1 cm. of the surface. It grew vigorously in the cooked meat medium with digestion of the meat particles, formation of black pigment, and generation of a characteristic rank odor. Spores formed readily and profusely in the meat medium.

BACILLUS TERTIUS

B. tertius was isolated in only three instances. As with *B. putrefaciens*, spore formation first appears as an oval terminal enlargement of the rod, which stains heavily. The fully developed spores were extremely large, of oval shape, and stained only very faintly. Growth in meat medium was accompanied by marked evolution of gas and decided pink coloration of the meat particles.

UNIDENTIFIED BACILLUS

An unidentified organism was commonly found in fresh hams. In form it is a very stout rod of approximately the same length as *B. sporogenes* but much thicker. It stains very unevenly. Even in young cultures areas which stain very faintly or not at all are observed. This is not due to spore formation but is suggestive of autolysis. It possesses numerous flagella but no motility was observed, although repeated observations were made. This organism is an extremely strict anaerobe. Spores form readily in the cooked meat medium. The spores are quite large, usually oval in shape but often show parallel sides and flattened ends. The peripheral outline of the spore stains densely. The spore seemingly forms centrally, but, when fully developed occupies nearly the entire rod with little, if any, distention. Occasionally at one end of the spore there is a slight projection of the rod. Glucose was fermented with production of acid and gas. Milk did not appear to be a favorable medium for growth. In cooked meat medium gas evolution was moderate, there was no appreciable decrease in the volume of meat particles, and no odor could be detected even in old cultures. This organism seemed to resemble *B. oedematiens*, but is not identified as such.

In this study particular attention has been given to *Bacillus putrefaciens* for the reason that it finds its optimum condition for growth at a lower temperature than any of the other organisms studied, and that it has been found so commonly associated with ham souring. The frequency with which it was found and isolated from fresh hams, together with the small size of the samples taken, justifies the belief that it is commonly present in fresh hams. Such being the case, it follows that the mere presence of *B. putrefaciens* in the ham is not sufficient to cause spoilage.

The first check applied in regular packing-house operations is that of refrigeration. Refrigeration is no doubt an effective check, but it must be remembered that organisms of the type studied are generally capable of growth at rather low temperatures. *Bacillus putrefaciens* in particular finds its most favorable growth temperature at from 20° to 25° C. (68° to 77° F.) and it grows readily, although less rapidly, at from 8° to 10° C. (46.5° to 50° F.). It is evident, therefore, that if refrigeration is to be effective it must needs be thorough. This is in harmony with the fact, well known in the meat-packing industry, that overtaking the capacity of the plant, particularly that of the refrigerating system, is likely to be followed by an "epidemic" of ham souring.

SUMMARY

Hams from dressed hog carcasses taken from the killing floor 45 minutes after slaughter were found to harbor microorganisms in the interior musculature, synovial fluid, and bone marrow. Similar organisms were found in hams from carcasses scalded in a steam cooker and scraped by hand, and in hams taken from the carcasses as soon as bleeding had been completed.

Five species of anaerobes, namely, *Bacillus putrefaciens*, *B. histolyticus*, *B. sporogenes*, *B. tertius*, and an unidentified organism resembling *B. oedematiens* in some respects, were isolated from such hams.

There were also present numerous and varied aerobic organisms.

No organisms of the *Bacillus coli* group were found, although a special search was made for them. It is concluded, therefore, that this group is rarely, if ever, present in the bacterial flora of fresh hams.

There is no evidence that any of the organisms found gained access through killing-floor operations.

CONCLUSION

From the foregoing data it is concluded that the organisms responsible for ham souring are disseminated throughout the carcass from the moment of slaughter and possibly are present in the blood and tissues of the living animal. It is evident, therefore, that access of these organisms can not be prevented by any alteration of killing-floor operations or practices. Prevention of ham souring depends, therefore, on preventing the development of the causative organisms which are known to be present. Prompt handling and prompt and efficient chilling resulting in early attainment of the low temperatures required to check the development of *Bacillus putrefaciens* and similar organisms, and maintenance of uniform low temperatures until sufficient salt has been taken up by the ham to hold these organisms in permanent check, are the means of prevention indicated. The effectiveness of these methods, is shown in a practical way by the low rate of spoilage attained by those establishments at which they are consistently practiced.

SEED TREATMENTS FOR SWEET-CORN DISEASES¹

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INTRODUCTION

This paper presents the data on sweet-corn seed treatments obtained in 1925 from 800 plots of 25 hills each. Approximately half of the plots were located near Bloomington, Ill., and the remainder at Ames, Iowa. Because of the rather promising economic possibilities of certain of the treatments, the results are thought to be worth publishing, even though the principal data are from experiments of only one year.

During the last five years experiments have been conducted on the treatment of dent-corn seed for the control of the seed-borne diseases. The results of these experiments are being published in another paper in which a limited survey of the pertinent literature is included. In the experiments with dent corn, the most promising results have been obtained during the past three years with organic compounds of mercury. It was anticipated that, as soon as the methods of control used in the dent-corn experiments were published, the sweet-corn growers would consider the advisability of applying these measures to seed-borne diseases of sweet corn. Therefore, rather extensive field experiments with sweet corn were conducted in 1925, in which the same materials and the same methods that had given the best results with dent corn were used.

Sweet corn and dent corn have many diseases in common, but in practically all instances the effects are more severe on sweet corn. The varieties of sweet corn of high quality in respect to sugar content are especially susceptible. This explains, in part, why it is so difficult to produce high-grade sweet-corn seed and why so much seed of inferior quality is used. It would be expected that any measures which would improve the quality of the seed would be more quickly adopted by sweet-corn growers than by dent-corn growers for the reason that the sweet-corn industry usually is more localized and, in general, is well organized.

In dent corn two diseases stand out in importance as influencing field stands and yields. These are the *Diplodia* disease, caused by *Diplodia zeae*, and the *Gibberella* disease, caused by *Gibberella saubinetii*. When seed lots badly infected with either or both of these organisms are planted, the resulting stands may vary from almost nothing to almost perfect stands, depending on the temperature and the moisture of the soil. Both diseases are destructive in sweet corn also, and somewhat promising results have been obtained

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in controlling them by the use of certain seed-treatment materials. However, there is another disease destructive to sweet corn but unimportant to dent corn, on which only limited data have been obtained. This is bacterial blight, or Stewart's disease, caused by *Aphanobacter stewartii*. This disease is more severe in the central Corn Belt than north of it, and most severe on varieties which naturally contain much sugar. Indications are that it is more difficult to control than either Diplodia or Gibberella disease.

EARLY EXPERIMENTS

In 1922, hot water was tried to some extent as a means of controlling seed-borne sweet-corn diseases. There was available for experimentation a composite of Minnesota Early Crosby sweet-corn ears which were highly infected with *Cephalosporium acremonium*, the cause of black-bundle disease. After being treated, the kernels were germinated in Petri dishes and examined with the compound microscope for the presence of the fungus. The data from this experiment are presented in Table 1. These data indicate that *C. acremonium* in sweet-corn seed may be killed by certain hot-water seed treatments. However, as it was considered unlikely that any hot-water method would be put into practical use, no further data were accumulated on this method.

TABLE 1.—Effects of hot-water treatments on Minnesota Early Crosby sweet-corn seed naturally infected with *Cephalosporium acremonium* at Madison, Wis., in 1922

Number of kernels	Presoak	Treatment		Infection	Germination	Condition
		Temperature	Minutes			
	Hours	° C.		Per cent	Per cent	
20.....	26	53	15	0	100	1 weak.
20.....	26	53	30	0	95	2 weak.
20.....	26	53	60	0	90	Several weak.
20.....	26	53	90	0	95	Do.
20.....	26	53	120	0	90	Do.
20.....	22	58	10	0	100	1 weak.
20.....	22	57	15	0	100	Several weak.
20.....	22	57	20	0	95	Do.
20.....	22	57	30	0	85	Do.
20.....	22	57	60	0	45	All weak.
20.....	21	63	10	0	5	Do.
20.....	21	63	15	0	0	Dead.
20.....	21	63	20	0	0	Do.
20.....	21	63	30	0	0	Do.
20.....	21	63	60	0	0	Do.
20.....	1	75	15	0	20	Very weak.
20.....	1	75	30	0	0	Dead.
20.....	1	75	60	0	0	Do.
20.....	1	75	120	0	0	Do.
20 (untreated control).....				80	95	

Table 2 presents the data obtained in a preliminary experiment with an organic mercury compound, chlorophol, used in a number of different concentrations and for varying lengths of time. Sweet corn from the same lot of Minnesota Early Crosby as that used in the hot-water treatments was employed.

The data presented in Table 2 indicate possibilities of controlling internal seed-borne infections of *Cephalosporium acremonium* with chlorophol liquid treatments. They also indicate that germination

is affected but little over a fairly wide range of concentrations and treatment periods. The results of this experiment suggested the advisability of trying this and similar compounds for the control of other internally seed-borne corn diseases.

TABLE 2.—Effects of chlorophol seed treatments on Minnesota Early Crosby sweet-corn seed naturally infected with *Cephalosporium acremonium*, at Madison, Wis., in 1922

Number of kernels	Strength of solution	Duration of treatment	Infection	Germination	Condition of seedlings			
					Strong	Medium	Weak	Dead
	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
20.....	0.25	1	0	95				
20.....	.5	1	0	100				
20.....	1.0	1	10	95				
20.....	2.0	1	0	90				
20.....	.25	2	0	100				
20.....	.5	2	0	95				
20.....	1.0	2	0	95				
20.....	2.0	2	0	100				
20.....	.25	3	0	90				10
20.....	.5	3	0	100	30	45	25	0
20.....	1.0	3	0	90	70	10	10	10
20.....	2.0	3	0	95	65	30	0	0
20.....	.25	5	0	95	50	40	5	5
20.....	.5	5	0	95	65	20	10	5
20.....	1.0	5	0	100	30	55	15	0
20.....	2.0	5	0	100	75	25	0	0
20 (untreated control)			80	95				

Table 3 shows the percentages of Minnesota Early Crosby sweet-corn plants diseased with bacterial blight in a field experiment in which the seed was treated for various periods of time with 0.25 per cent solutions of chlorophol. The presence of the disease was determined by cutting up the plants when they were about half grown and noting whether or not they had the yellow vascular bundles with the characteristic bacterial ooze.

The data in Table 3 show that treatment of Minnesota Early Crosby sweet corn with 0.25 per cent chlorophol solution during periods up to 24 hours does not decrease appreciably the amount of infection in the resulting plants.

TABLE 3.—Percentages of bacterial blighted plants in Minnesota Early Crosby sweet corn grown from seed treated during different periods of time with chlorophol (0.25 per cent soak), at Bloomington, Ill., in 1923

Duration of treatment in hours	Infected plants in—			
	Series 1		Series 2	
	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>
0.....	21	42	12	48
6.....	20	40	12	48
9.....	29	58	11	44
12.....	30	60	14	56
20.....	23	46	11	44
24.....	27	54	19	76

Table 4 presents data on field stand and presence of bacterial blight in two lots of Golden Bantam sweet corn the seed of which was treated for various periods of time with 0.25 per cent solutions of

chlorophol. Lot 1 consisted of a selection of ears of Golden Bantam sweet corn grown in Connecticut. Lot 2 was a bulk sample bought on the open market in Illinois. The results were recorded when the corn was in the canning stage. As in the preceding experiment, the bacterial infections were determined by cutting open the stalks.

The data in Table 4 agree with those in Table 3 in that no practical control of bacterial blight of sweet corn was obtained by treating the seed in 0.25 per cent solutions of chlorophol.

The data also show that the stands from these two lots of Golden Bantam seed were, with one exception, increased by the shorter treatments and injured by the longer treatments.

TABLE 4.—*Data on field stand and bacterial blight of plants grown from two lots of Golden Bantam sweet-corn seed treated with chlorophol (0.25 per cent soak), at Bloomington, Ill., in 1925*

Stand from untreated seed	Duration of treatment	Stand			Bacterial blighted plants	
		Treated seed	Increase over		Un-treated	Treated
			Nearest control	Average of controls		
	Hours	Per cent	Per cent	Per cent	Per cent	Per cent
Seed Lot No. 1:						
82 per cent.	6	90	9.8	12.5	No data.	No data.
83 per cent.	8½	87	4.8	8.8	No data.	No data.
89 per cent.	12	83	20.3	3.8	19.0	11.4
83 per cent.	20	76	-8.4	-5.0	29.0	23.6
83 per cent.	24	75	-9.6	-6.3	14.9	12.1
Seed Lot No. 2:						
65 per cent.	6	80	23.1	24.2	No data.	No data.
66 per cent.	8½	80	-9.1	-6.8	No data.	No data.
55 per cent.	12	70	27.3	8.7	No data.	No data.
65 per cent.	20	50	-23.1	-22.4	22.5	16.7
71 per cent.	24	53	-25.4	-17.7	27.3	16.1

Average stand from untreated seed: Lot No. 1, 80; Lot No. 2, 64.4.

EXPERIMENTS IN 1925

The purpose of the experiments made in 1925 was to determine the value of such materials and methods as already had proved most beneficial in the control of certain dent-corn diseases.

The results of the seed treatments with sweet corn and also with dent corn have been previously summarized.³

For the sweet-corn experiments, six materials were chosen. Three of these were used as water solutions, in which the sweet-corn kernels were immersed for an hour and a half, and three were used as dusts. Six lots of seed were used in which three varieties were represented. These were (1) Country Gentleman, nearly disease-free, Diplodia-infected, and Gibberella-infected; (2) Canadian Evergreen, nearly disease-free and diseased; and (3) Golden Delicious, diseased.⁴ Of these six lots, two were good seed, being nearly disease-free. The diseased lots of Canadian Evergreen and Golden Delicious were of bulk seed obtained from representative growers, but which would

³ REDDY, C. S., HOLBERT, J. R., and ERWIN, A. T. SWEET CORN SEED TREATMENT IN 1925. (Abstract) Phytopathology 16: 65. 1926.

HOLBERT, J. R., REDDY, C. S., and KOEHLER, B. SEED TREATMENTS FOR THE CONTROL OF CERTAIN DISEASES OF DENT CORN. (Abstract) Phytopathology 16: 82-83. 1926.

⁴ The seed of the first variety was supplied by the Bloomington (Illinois) Canning Company, and the seed of the other two varieties by G. N. Hoffer.

be classified by them as somewhat inferior. The two diseased lots of Country Gentleman were ear selections from the germinator and composited, so that one contained a high percentage of kernels infected with *Diplodia zeae*, one of the dry-rot organisms, and the other was highly infected with *Gibberella saubinetii*, one of the root-rot organisms. These two diseased composites germinated 95 and 85 per cent, respectively. In macroscopic appearance, they differed very little from the nearly disease-free lot. However, they were more highly diseased than seed usually used for planting. Only in certain years would they be comparable to any considerable proportion of commercial seed. They were included in these experiments mainly for the purpose of determining the effect of seed treatments on the control of specific diseases. From the results of seed-treatment experiments on these six lots, it was thought that one could learn, to some extent, the effect of the treatments (1) on different varieties, (2) on nearly disease-free seed and on diseased bulk seed of certain varieties, and (3) on the control of specific seed-borne diseases. It would be expected that the results from bulk seed would correspond closely to what would be experienced under similar environmental conditions by practical growers if they adopted the same methods of treatment.

The treatments in all the experiments were made at one place, at one time, and at one temperature (25° C.). All liquid treatments were for 1½ hours in solutions of 0.4 per cent strength, except Corona 620, which was of 0.25 per cent strength. The water-soak treatment also was for 1½ hours. The seed was dried immediately after treating by spreading on screens in thin layers. Each dust was applied by shaking the seed and the dust vigorously in a closed vessel and sifting out the excess dust.

The same planting method was used in Illinois and Iowa, but 10 replications of each treatment and control were made in the Illinois experiment, while the space provided for only seven replications in the Iowa experiment. Each replication was a row consisting of 25 hills planted exactly three kernels to the hill. Both hills and rows were spaced 3½ feet apart. The six lots were placed side by side, making the experiment in Illinois 80 by 150 hills (more than 3 acres in size), and in Iowa 56 by 150 hills.

TABLE 5.—Mean field stands of sweet-corn varieties from untreated and treated seed, in plots replicated 10 times in Illinois and 7 times in Iowa, 1925

Treatment	Country Gentleman						Canadian Evergreen				Golden Delicious	
	Nearly disease-free		Diplodia-infected		Gibberella-infected		Nearly disease-free		Diseased		Diseased	
	Illinois	Iowa	Illinois	Iowa	Illinois	Iowa	Illinois	Iowa	Illinois	Iowa	Illinois	Iowa
No treatment.....	P. ct. 86.8	P. ct. 92.4	P. ct. 38.8	P. ct. 51.9	P. ct. 54.7	P. ct. 68.2	P. ct. 83.4	P. ct. 92.3	P. ct. 78.5	P. ct. 82.9	P. ct. 52.0	P. ct. 49.5
Water soak (1½ hours).....	85.4	91.4	40.2	53.0	53.0	72.0	83.7	96.1	74.6	84.0	54.0	46.3
Semesan soak (1½ hours).....	86.0	92.9	62.4	64.1	65.9	72.9	83.5	91.1	73.3	89.9	62.0	63.5
Semesan Junior (dust).....	85.6	90.8	61.4	59.2	62.4	74.6	81.5	88.0	74.6	84.4	56.0	62.8
Uspulun soak (1½ hours).....	85.2	91.5	68.2	62.9	64.4	72.1	87.9	92.6	81.4	87.4	63.0	69.7
Bayer Dust.....	86.8	91.8	62.0	68.3	65.7	72.7	86.4	91.5	73.8	87.2	57.0	58.7
Corona 620 soak (1½ hours).....	86.6	89.3	64.4	63.8	70.8	72.0	82.4	86.1	68.8	76.5	58.5	65.1
Corona 640 soak (dust).....	86.8	90.6	62.9	68.3	61.3	75.5	85.4	85.4	79.4	85.6	60.0	62.5

Field stand data were taken when the plants were 6 inches to 1 foot high. The production data were recorded as total yields and yields of good corn. Total yields of plots were obtained by weighing the snapped ears without removing the husks. Yields of good corn were obtained, after the husks were removed, by weighing only the ears prime for canning purposes. Yield of good corn, therefore, is the weight of husked ears after discarding nubbins, rotten ears,

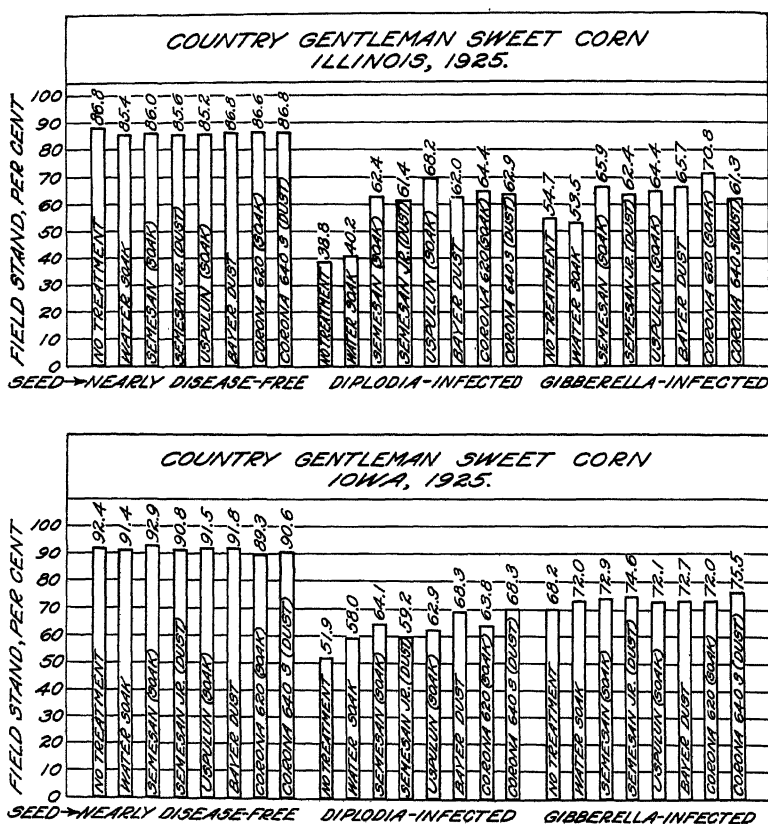


FIG. 1.—Graph showing mean field stands of sweet corn from untreated and treated seed, in plots replicated 10 times in Illinois and 7 times in Iowa, 1925. (Data in Table 5)

and ears which had not reached or had passed the prime stage. The field stand data are presented in Table 5 and Figure 1.

The data in Table 5 and Figure 1 show that the stands resulting from good (nearly disease-free) seed are affected but little by the treatments used in the experiments. The stands from diseased seed, with some exceptions in one lot, are increased materially. The percentage of increase in each case probably depended on such factors as the particular diseases present in the diseased lot, the percentage of kernels infected, the conditions under which germination and early growth took place, and others. It is known in a general way

from previous experiments that *Diplodia*-infected and *Gibberella*-infected dent-corn seed give poorer stands when planted in cold soil than in warm, and in reasonably wet soil than in dry. In line with this, the data show poorer stands in the Illinois than in the Iowa experimental plots. The plots in Illinois were planted May 18 and 19 in cool, dry soil, while those in Iowa were planted May 28 in somewhat warmer, dry soil. It is to be expected that if a treatment controlled a certain seed-borne disease of this type, without injury to the seed, the greatest differences between stands in treated and untreated plots would occur when plantings were made under conditions most favorable for the disease. As conditions in Illinois at planting time were more favorable to *Diplodia* dry-rot and *Gibberella* root-rot diseases than were those in Iowa, greater differences in field stands from treated and untreated infected seed were recorded in the Illinois than in the Iowa experiments.

Diplodia seedling blight as compared with *Gibberella* seedling blight seems somewhat more amenable to control by certain seed treatments. This explains to some extent why there are greater differences in stands from treated and untreated *Diplodia*-infected seed than from treated and untreated *Gibberella*-infected seed. The mean field stands from treated *Diplodia*-infected seed were 63.8 per cent greater at Illinois and 24.1 per cent greater at Iowa than those of the respective controls, while the corresponding differences between treated and untreated *Gibberella*-infected seed were only 19.0 and 7.5 per cent, respectively.

TABLE 6.—*Total yields and yields of prime canning sweet corn in pounds per acre at canning stage from seed untreated, seed soaked in water for 1½ hours, seed treated with Semesan Jr., Bayer, and Corona 640 S dusts, and seed treated by soaking for 1½ hours in 0.5 per cent solutions of Semesan and Uspulun and 0.25 per cent solutions of Corona 620. Grown near Bloomington, Ill., and at Ames, Iowa, in 1925*

Variety	Seed condition	Number of repli- ca- tions	No treatment		Water soak		Semesan (soak)		Semesan Jr. (dust)	
			Total	Good	Total	Good	Total	Good	Total	Good
Illinois experi- ments:										
Country Gentle- man.	Nearly disease-free	10	3,740	3,600	3,800	3,628	3,864	3,728	3,963	3,827
Do.	<i>Diplodia</i> -infected	10	2,390	2,232	2,270	2,118	3,535	3,326	3,411	3,229
Do.	<i>Gibberella</i> -infected *	10	2,360	2,231	2,460	2,316	2,948	2,800	2,892	2,757
Canadian Evergreen.	Nearly disease-free	10	3,710	3,454	3,666	3,397	3,963	3,724	4,011	3,724
Do.	Diseased	10	3,059	2,895	3,023	2,483	3,178	3,009	2,812	2,653
Golden De- licious.	Diseased *	10	2,390	2,160	2,454	2,188	2,692	2,473	2,572	2,359
Iowa experi- ments:										
Country Gentle- man.	Nearly disease-free	7	4,592	3,719	4,450	3,455	4,734	3,658	4,511	3,658
Do.	<i>Diplodia</i> -infected	7	2,540	1,605	3,028	2,011	3,150	2,215	2,824	2,052
Do.	<i>Gibberella</i> -infected *	7	5,141	3,962	5,304	4,328	4,917	4,023	5,344	4,287
Canadian Evergreen.	Nearly disease-free	7	5,940	4,690	5,235	4,495	5,432	4,495	4,926	3,985
Do.	Diseased	7	6,035	5,161	5,486	4,816	6,157	5,263	6,157	5,263
Golden De- licious.	Diseased *	7	2,967	2,520	2,479	2,134	3,495	3,170	3,658	3,069

* Bayer compound 0.4 per cent (soak) was used instead of Corona 620.

TABLE 6.—Total yields and yields of prime canning sweet corn in pounds per acre at canning stage from seed untreated, seed soaked in water for $1\frac{1}{2}$ hours, seed treated with Semesan Jr., Bayer, and Corona 640 S dusts, and seed treated by soaking for $1\frac{1}{2}$ hours in 0.5 per cent solutions of Semesan and Uspulun and 0.25 per cent solutions of Corona 620. Grown near Bloomington, Ill., and at Ames, Iowa, in 1925—Continued

Variety	Seed condition	Number of replications	Uspulun (soak)		Bayer (dust)		Corona 620 (soak)		Corona 640 S (dust)	
			Total	Good	Total	Good	Total	Good	Total	Good
Illinois experiments:										
Country Gentleman.	Nearly disease-free	10	3, 670	3, 557	3, 626	3, 444	3, 530	3, 401	3, 608	3, 472
Do.	Diplodia-infected	10	3, 626	3, 439	3, 063	2, 900	3, 151	2, 986	3, 070	2, 913
Do.	Gibberella-infected ^a	10	2, 956	2, 785	2, 807	2, 658	2, 690	2, 544	2, 511	2, 316
Canadian Evergreen.	Nearly disease-free	10	3, 847	3, 706	3, 525	3, 297	3, 652	3, 454	3, 785	3, 397
Do.	Diseased	10	3, 080	2, 952	3, 022	2, 824	2, 690	2, 511	3, 057	2, 852
Golden Delicious.	Diseased ^a	10	3, 074	2, 886	2, 493	2, 274	2, 534	2, 260	2, 376	2, 188
Iowa experiments:										
Country Gentleman.	Nearly disease-free	7	4, 308	3, 272	4, 592	3, 455	4, 088	3, 374	4, 267	3, 455
Do.	Diplodia-infected	7	3, 332	2, 093	3, 515	2, 560	3, 028	2, 032	3, 150	2, 194
Do.	Gibberella-infected ^a	7	4, 857	3, 789	5, 121	4, 267	5, 161	4, 145	5, 222	4, 470
Canadian Evergreen.	Nearly disease-free	7	5, 865	4, 887	5, 432	4, 651	5, 239	4, 377	4, 870	4, 026
Do.	Diseased	7	5, 832	5, 181	6, 015	5, 283	5, 364	4, 531	5, 670	4, 917
Golden Delicious.	Diseased ^a	7	3, 902	3, 496	3, 394	3, 068	3, 983	3, 414	3, 211	2, 825

^a Bayer compound 0.4 per cent (soak) was used instead of Corona 620.

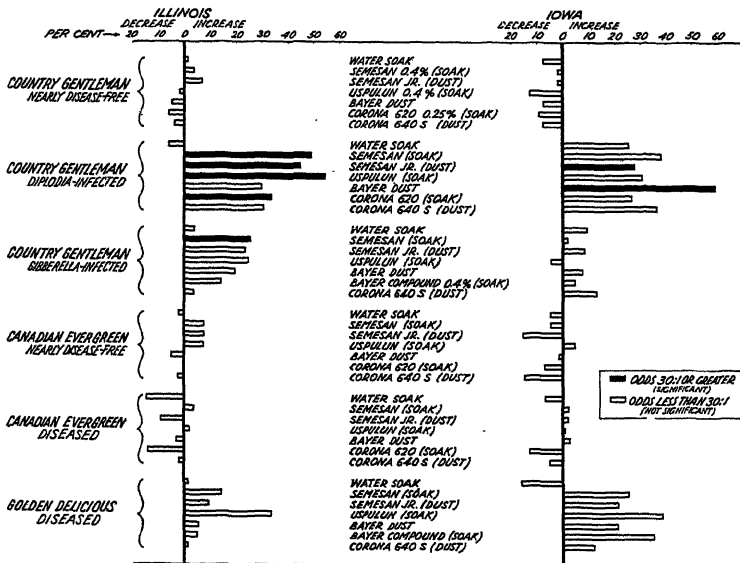


FIG. 2.—Graph showing percentage increases and decreases in yields from various lots of nearly disease-free and diseased sweet-corn seed, following certain seed treatments at Bloomington, Ill., and Ames, Iowa, 1925

The yield data from the Illinois and Iowa plots are presented in Table 6 and summarized in Table 7 and Figure 2. These data show

that yields from nearly disease-free seed are not significantly changed by certain seed treatments, and that some lots of diseased sweet-corn seed are benefited more than others. The most noticeable effects produced by the treatments were on *Diplodia*-infected and *Gibberella*-infected seed.

TABLE 7.—Summary of data in Table 6 showing percentage increases (+) or decreases (—) in yields of sweet corn prime for canning (odds by "Student's" Method)

Treatment and location	Country Gentleman						Canadian Evergreen						Golden Delicious		All lots
	Nearly disease-free		Diplodia-infected		Gibberella-infected		Nearly disease-free		Diseased		Diseased				
Water soak:	Per cent	Odds	Per cent	Odds	Per cent	Odds	Per cent	Odds	Per cent	Odds	Per cent	Odds	Per cent	Odds	
Illinois.....	+0.8	1:1	-5.1	2:1	3.8	2:1	-1.7	1:1	-14.2	18:1	1.3	1:1	-2.7		
Iowa.....	-7.1	3:1	25.3	9:1	9.2	8:1	-4.2	3:1	-6.7	3:1	-15.3	6:1	-1.9	4:1	
Semesan, 0.4 per cent (soak):															
Illinois.....	3.6	2:1	49.0	1,427:1	25.5	132:1	7.8	4:1	3.9	2:1	14.5	4:1	15.0	-----	
Iowa.....	-1.6	1:1	38.0	22:1	1.5	1:1	-4.2	2:1	2.0	2:1	25.8	9:1	5.4	7:1	
Semesan Jr. (dust):															
Illinois.....	6.3	3:1	44.7	344:1	23.6	20:1	7.8	3:1	-8.4	7:1	9.2	4:1	11.9	-----	
Iowa.....	-1.6	2:1	27.9	31:1	8.2	6:1	-15.0	5:1	2.0	2:1	21.8	8:1	3.0	3:1	
Uspulun, 0.4 per cent (soak):															
Illinois.....	-1.2	1:1	54.1	908:1	24.8	10:1	7.3	5:1	2.0	1:1	33.6	29:1	16.6	-----	
Iowa.....	-12.0	10:1	30.4	14:1	-4.1	3:1	4.2	2:1	0.4	1:1	38.7	12:1	4.9	5:1	
Bayer Dust:															
Illinois.....	-4.3	1:1	29.9	19:1	19.1	10:1	-4.5	2:1	-2.5	1:1	5.3	1:1	5.0	-----	
Iowa.....	-7.1	4:1	59.5	79:1	7.7	5:1	-0.8	1:1	2.4	2:1	21.7	7:1	7.5	13:1	
Corona 620, 0.25 per cent (soak):															
Illinois.....	-5.5	2:1	33.8	50:1	14.0	5:1	0.0	-----	-13.3	18:5	4.6	1:1	3.5	-----	
Iowa.....	-9.3	5:1	28.6	8:1	4.6	4:1	-6.7	3:1	-12.2	22:1	35.5	26:1	1.0	-----	
Corona 640 S. (dust):															
Illinois.....	-3.6	2:1	30.5	11:1	3.8	1:1	-1.7	1:1	-1.5	1:1	1.3	1:1	3.4	-----	
Iowa.....	-7.1	11:1	36.7	22:1	12.8	11:1	-14.2	8:1	-4.7	3:1	12.1	4:1	1.1	1:1	

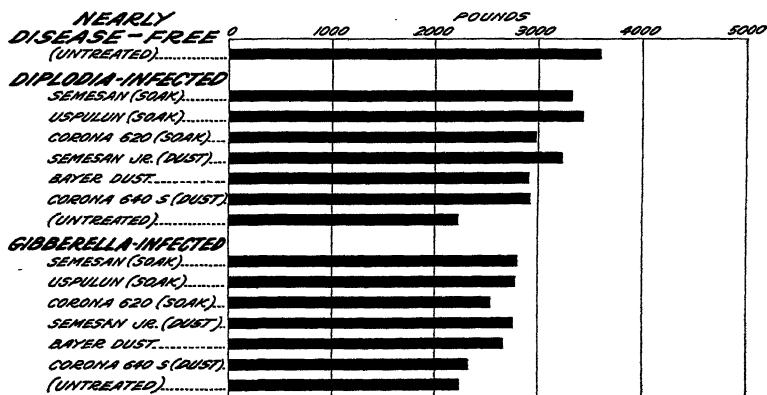


FIG. 3.—Graph showing yields of Country Gentleman sweet corn prime for canning, from nearly disease-free seed and from treated and untreated *Diplodia*-infected and *Gibberella*-infected seed at Bloomington, Ill., 1925

Figures 3 and 4, which are graphical presentations of data from Table 6, show acre yields from three composites of Country Gentleman sweet-corn seed of the same strain. Figure 3 presents the results from the Illinois plots which were on land that might be

considered uniform, not only for the plots in a given lot but also for the different lots. The Iowa plots were uniform for the plots in each lot, but not uniform for the different lots. Hence, Figure 3 represents the yields of lots grown on uniform soil where differences are largely due to the presence or absence of disease in the lots and to the effectiveness of the seed treatments. In like comparisons of yields from the Iowa plots, shown in Figure 4, the differences between the lots are due largely to differences in soil fertility.

The purpose of Figure 3 is to present the available data pertaining to relative yields from untreated, nearly disease-free seed and from untreated and treated diseased seed of the same strain. Therefore, this graph indicates the value of good seed as compared with that of certain types of diseased seed, whether untreated or treated. Figure 3 shows that the untreated *Diplodia*-infected seed yielded 38 per cent less than the untreated nearly disease-free seed, but the best yield from treated *Diplodia*-infected seed was only 4.5 per cent less than that from the untreated nearly disease-free seed. The value of

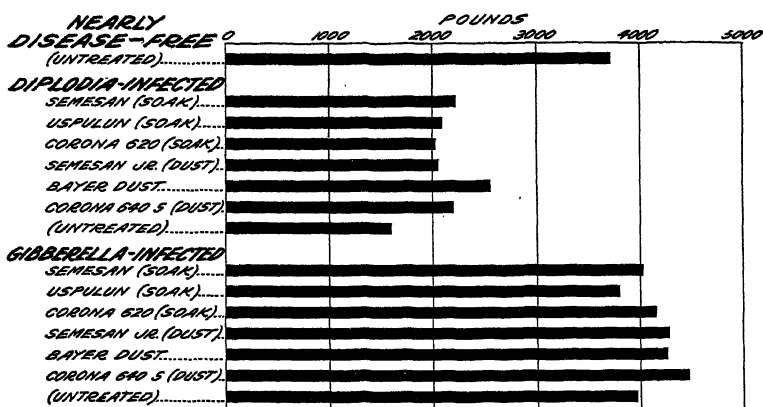


FIG. 4.—Graph showing yields of Country Gentleman sweet corn prime for canning, from nearly disease-free seed and from treated and untreated *Diplodia*-infected and *Gibberella*-infected seed at Ames, Iowa, 1925

seed treatment in this case was relatively large because the diseased seed lot had but few dead kernels and, excluding the dry-rot disease, was as free from certain types of weakness as was the nearly disease-free lot.

Figure 3 shows that untreated *Gibberella*-infected seed also yielded 38 per cent less than the untreated nearly disease-free seed, but the best yield from treated *Gibberella*-infected seed was still 22.2 per cent less than the yield from nearly disease-free seed. The value in this case was less, not necessarily because the *Gibberella* disease is more difficult to control than the *Diplodia* disease, but probably because the seed lot contained 15 per cent of dead kernels, upon which seed treatments have no practical effect.

DISCUSSION

The results of experiments herein reported show that treatment of sweet-corn seed with any of several different organic mercury compounds largely prevents the seedling blights caused by *Diplodia*

zeae and *Gibberella saubinetii* and does not injure the seed. The two diseases produced by these organisms usually are the most important of the fungous diseases which cause unsatisfactory field stands. These diseases are widespread and are found to some extent in most sweet-corn seed. In certain years, sweet-corn seed generally is highly infected. In other years, severe infection of seed occurs only in some sections. Therefore, at least at times, treating the seed would be advantageous in the economic production of sweet corn.

Many may follow the practice of treating sweet-corn seed without knowing whether or not either of these two diseases is present. Especially for them, it may be well to point out more definitely the limits of sweet-corn seed treatments in order to prevent expectation of results beyond those indicated by the data.

Seed treatments can not replace satisfactorily any of the methods now used in obtaining good sweet corn, and should not interfere in any way with any line of sweet-corn improvement. As shown by the data, the treatments do not put life into dead seeds, and it is not expected that they will materially affect those weaknesses of the seed caused by immaturity, early fall frosts, and exposures to low temperatures when the moisture content of the seed is high.

Seed treatments of sweet corn will be of most value in those years when it is practically impossible to obtain seed that is not somewhat heavily infected with *Diplodia zeae* and *Gibberella saubinetii*.

SUMMARY

No practical control of bacterial blight of sweet corn was obtained by treating the seed.

When nearly disease-free seed was used, the field stands and yields were affected but little by seed treatments with organic mercury compounds.

When seed diseased with *Diplodia zeae* or *Gibberella saubinetii* was used, the field stands and yields nearly always were materially increased by seed treatments with organic mercury compounds.

The yields from nearly disease-free seed were slightly higher than those from treated *Diplodia*-infected seed, and considerably higher than those from treated *Gibberella*-infected seed.

When only diseased seed is available for planting, certain seed treatments will have distinct value. Such seed treatments do not injure the seed.



CORRELATIONS OF SEED, FIBER, AND BOLL CHARACTERS IN COTTON¹

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INTRODUCTION

The purpose of this contribution is to bring together in convenient form information in regard to the correlations existing among the characters of the bolls, seeds, and fiber of cotton. Numerous determinations have been made at the United States field station, Sacaton, Ariz., chiefly on Pima and other varieties of the Egyptian type, but also on a second generation population of a hybrid between upland and Egyptian cottons. The data obtained in Arizona are supplemented by reference to the published data of other investigators who worked chiefly with upland cottons.

With the exception of the above-mentioned hybrid, the material on which the correlations were determined was more or less homogeneous, hence not suitable for revealing such linkages as may exist among the characters considered. It was sought to ascertain the physical and physiological interrelations of characters that are of practical importance in cotton breeding, but the data thus obtained should be useful for comparison in future genetic studies.

All correlations were determined by the product-moment method.

CORRELATIONS DETERMINED ON INDIVIDUAL BOLLS OF PIMA COTTON

The population comprised 50 plants scattered through two plots of the commercial stock of Pima cotton at Sacaton in 1925. Ten characters were determined on from two to five bolls on each plant, only 3-lock bolls being used. Boll length, boll diameter, and boll index were determined on 250 bolls and the other characters on 224 bolls. Each boll was tagged when measured. The external dimensions were measured on bolls judged to be fully developed although still closed. The length and the maximum diameter of the boll were measured with specially designed calipers (figs. 1 and 2). To check the first measurements, many of the bolls were measured again two weeks later, but as the second series was incomplete, the first measurement was used in plotting the correlations, except in 14 cases where the second measurement showed a noteworthy increase in one or both dimensions. When these bolls had opened naturally the dry seed cotton was gathered and was weighed on a chemical balance. The content of each boll was then ginned separately and the weight of the seeds was determined. The weight of the fiber was determined by subtraction.²

¹ Received for publication July 7, 1926; issued October, 1926.

² The determination of all characters were made by George J. Harrison, Robert H. Peebles, and Dow D. Porter.

All correlations were plotted on the basis of determinations on the same individual bolls; hence the number was in each case 224, except

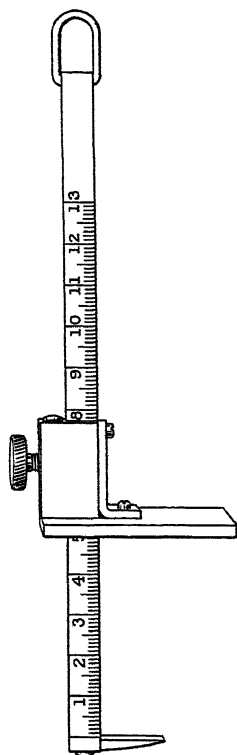


FIG. 1.—Caliper (designed by W. G. Wells) for measuring the length of cotton bolls without detaching them from the plant. The fixed arm is placed so that the base of the boll rests upon its thin edge and the adjustable plate is moved along the vertical bar (scaled in millimeters) until it rests lightly upon the tip of the boll. The screw is then tightened and the reading is taken.

in the correlations of boll length with boll diameter, boll length with boll index, and boll diameter with boll length, in which the number was 250. Computations by the product-moment method were made of all possible correlations of the nine characters seed cotton weight per boll, fiber weight per boll, lint percentage, lint index, number of seeds per boll, mean weight of the individual seeds, boll length, boll diameter, and boll index. The coefficients of correlation are given in Table 1.

Seed-cotton weight per boll shows, as would be expected, very high positive correlations with fiber weight per boll and number of seeds per boll. It is also positively and very significantly but not very highly correlated with the external length and diameter of the boll, hence it may be concluded that these external measurements of the fully developed but unopen boll give only a fair indication of the weight of its matured contents. Still lower are the positive correlations of seed cotton weight with lint

percentage and lint index, yet both are significant (r/E 5.8 and 9.0). The occurrence of positive correlations in these cases probably signifies that favorable conditions, conducive to the development of heavy bolls, are also conducive to a greater increase in the weight of fiber than of seed.

Fiber weight per boll is similar to seed-cotton weight in its correlations with other characters. The high positive correlation of fiber weight with number of seeds per boll shows the importance of the degree of fertilization as a factor in the yield of cotton (6;

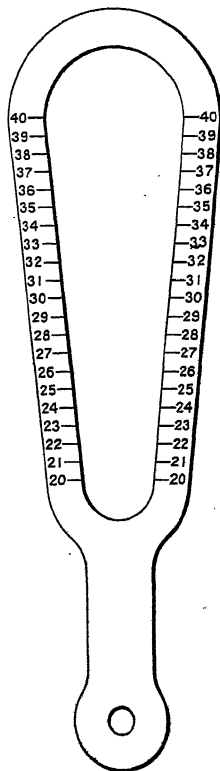


FIG. 2.—Caliper (designed by E. C. Chilcott and L. J. Briggs) for measuring the diameter of cotton bolls without removing them from the plant. The angle formed by the diverging sides of the loop and the calibration of the intervals (millimeters) marked on them are so calculated that when the loop is placed horizontally over the boll at its point of greatest diameter and is pushed forward (from the handle) as far as possible without compressing the boll, the maximum diameter of the boll is indicated by the figure on the scale at the point with which the side of the boll is in contact.

7, p. 49-50).^{3,4} As would be expected, fiber weight is more closely correlated than seed-cotton weight with lint percentage and lint index.

TABLE 1.—Correlations of seed, fiber, and boll characters determined on 224 or 250 individual bolls from 50 plants of Pima cotton, Sacaton, 1925

	Seed cotton weight per boll	Fiber weight per boll	Lint percentage	Lint index	Number of seeds per boll	Mean weight of individual seeds	Boll length	Boll diameter	Boll index
Seed cotton weight per boll		0.858± .012	0.244± .042	0.352± .039	0.757± .019	0.105± .045	0.418± .037	0.546± .032	0.008± .045
Fiber weight per boll	0.858± .012		.602± .029	.542± .032	.700± .023	-.104± .045	.415± .037	.445± .036	.093± .045
Lint percentage ^a	.244± .042	.602± .029		.540± .032	.284± .041	-.432± .036	.179± .044	.074± .045	-.142± .044
Lint index ^b	.352± .039	.542± .032	.540± .032		-.073± .045	.379± .038	.237± .042	.228± .042	-.100± .045
Number of seeds per boll	.757± .019	.700± .023	.284± .041	-.073± .045		-.433± .036	.310± .041	.380± .038	-.041± .045
Mean weight of individual seeds ^c	.105± .045	-.104± .045	-.432± .036	.379± .038	-.433± .036		.059± .045	.189± .043	.076± .045
Boll length	.418± .037	.415± .037	.179± .044	.237± .042	.310± .041	.059± .045		.385± .038	.680± .023
Boll diameter	.546± .032	.445± .036	.074± .045	.228± .042	.380± .038	.189± .043	.385± .038		.325± .040
Boll index ^d	.008± .045	.093± .045	-.142± .044	-.100± .045	-.041± .045	.076± .045	-.680± .023	.325± .040	

^a Weight of fiber as a percentage of the weight of seed cotton.

^b Weight of fiber \times 100 divided by the number of seeds.

^c Weight of seeds divided by number of seeds.

^d Maximum diameter as a percentage of the length.

Lint percentage shows a positive and very significant but far from perfect correlation with lint index, and we may conclude that the weight of the seeds is almost if not quite as important as the abundance of the fiber in determining the lint percentage (1, 5). The occurrence of a positive correlation (r 0.284) between lint percentage and number of seeds per boll, with a coefficient seven times its probable error, might be regarded as a consequence of the positive correlation of similar magnitude between lint percentage and weight of seed cotton per boll, the latter character in turn being highly positively correlated with number of seeds per boll. The partial correlation of lint percentage with number of seeds *constant* for weight of seed cotton gives, however, a coefficient that is still significant (r 0.156 \pm 0.044), indicating that there is some association of high lint percentage with a large number of seeds, independent of the other correlations mentioned and in spite of the fact that in this population weight of fiber per seed (lint index) is not significantly correlated with number of seeds.

³ Reference is made by number (italic) to "Literature cited," p. 796.

⁴ Counts of the number of ovules in 3-celled ovaries of Pima cotton, made at Sacaton in 1921 on 250 ovaries, showed a range of from 15 to 24, and a mean of 20.5 \pm 0.09 (7, p. 51). Since determinations at two localities in 1922 gave means of 20.8 and 21.5, it may be assumed that the true mean number of ovules in 3-celled ovaries of this variety is 21. The 224 three-lobed bolls of Pima cotton in 1925 showed a range of from 8 to 23 seeds per boll, the mean having been 17.6 \pm 0.13. This mean indicates that 84 per cent of the ovules had been fertilized under the exceptionally favorable conditions for cotton pollination prevailing at Sacaton. As would be expected, there was greater variation in the number of seeds than in the number of ovules, the standard deviations having been, for seeds in 1925, 2.853 \pm 0.091 and for ovules in 1921, 2.180 \pm 0.066. The difference amounts to 31 per cent and is six times its probable error.

Lint percentage shows a fairly high and very significant negative correlation with mean weight of the seeds. The lint percentage is not significantly correlated with boll diameter, but with boll length it shows a slight but probably significant positive correlation (r/E 4.1) which doubtless accounts for the low negative correlation of lint percentage with boll index (r/E 3.2).

Lint index, unlike the lint percentage, is not significantly correlated in this material with number of seeds per boll, hence it may be concluded that the weight of the fiber borne by the individual seed is not affected by the number of seeds in the boll. On the other hand, there is a fairly high and very significant positive correlation between lint index and mean weight of the individual seeds.⁵ There are significant positive correlations of nearly equal magnitude between lint index and the external length and diameter of the boll (r/E 5.6 and 5.4). In other words, the larger bolls tend to have a greater abundance of fiber on the individual seeds, but why this relation obtains is not clear. Unless there is a linkage involved, it probably expresses merely a like effect of environmental conditions on characters that are entirely independent.

Number of seeds per boll, in addition to the correlations already mentioned, shows a fairly high and very significant negative correlation with mean weight of the individual seeds, and is positively and very significantly correlated with boll length and boll diameter (r/E 7.6 and 10.0).

Boll length is positively and very significantly correlated with boll diameter; in other words, large bolls tend to be large in both dimensions and vice versa. A higher positive correlation than 0.385 might have been expected.

Boll index, an expression of the shape of the boll, shows no correlation with other characters except the expected ones with its components boll length and diameter, the unimportance of the low negative correlation with lint percentage having been pointed out.

It was thought possible that the external length and diameter of the boll when integrated would show a higher positive correlation with weight of the contents (seed-cotton weight) than either external measurement taken separately. The correlations of seed-cotton weight with boll length plus the diameter and boll length plus twice the diameter, therefore were computed, and the values found for r were 0.518 ± 0.033 and 0.582 ± 0.030 . Neither coefficient differs significantly from that of the correlation seed-cotton weight with boll diameter (r 0.546 ± 0.032).

The correlation between lint index and grade of seed fuzziness also was computed, and gave a value for r of only 0.042 ± 0.045 .⁶ Very smooth seeded varieties of cotton tend to have sparse lint and the same appears to be true in general of such exceptionally smooth seeded individuals of the Pima variety as grade only 1 or 2 in seed

⁵ The reality of this correlation is shown by the fact that when both fiber weight per boll and number of seeds per boll are held constant, the coefficient of partial correlation is 0.244 ± 0.042 (r/E 5.8), which is not significantly lower than the coefficient of the original correlation of lint index with mean weight of the seeds (r 0.379 ± 0.038).

⁶ This correlation was determined in Arizona also on 180 F₃ plants of an Upland-Pima hybrid in 1919, on 178 plants of Pima cotton, in 1922, and on 36 plants of Sakellaridis cotton in 1922. In no case was the coefficient significant.

fuzziness.⁷ But the 50 plants of the commercial stock of Pima which furnished the material for this correlation all had more or less fuzzy seeds (grades 4 to 9), and within a population of a single variety having this range of fuzziness there appears to be no relation between the abundance of short fibers (fuzz) and of long fibers (lint).

COMPARISON OF CORRELATIONS BASED ON INDIVIDUAL BOLLS AND ON THE MEANS OF SAMPLES CONSISTING OF NUMEROUS BOLLS

Correlations of eight pairs of characters were determined in 1925 on two lots of bolls of the commercial stock of Pima cotton: (A) 224 individual bolls borne on 50 plants growing in the same field at Sacaton and (B) 25 samples from as many fields in the Salt River Valley, each sample comprising 100 bolls, each boll from a different individual plant. For the 224 bolls (series A) as mentioned in the preceding section, each character was determined separately on each individual boll. For the 25 samples (series B) the mean seed cotton weight, fiber weight, and number of seeds per boll were obtained by dividing the total weight or number in the sample by 100. Lint percentage was obtained by dividing the total fiber weight by the total seed cotton weight of the sample. Lint index was obtained by multiplying the total fiber weight of the sample by 100 and dividing by the total number of seeds. Mean weight of the individual seeds is the total weight of seeds in the sample divided by the total number of seeds.

Table 2 gives, for both populations, the statistical constants of the characters involved in the correlations. The coefficients of correlation are given in Table 3.

TABLE 2.—*Statistical constants of the characters involved in correlations determined on (A) 224 individual bolls of Pima cotton and on (B) 25 samples of 100 bolls each of Pima cotton*^a

Character	Mean	Standard deviation of the mean
Seed cotton weight per boll (grams).....	A 3.15 ± 0.023 B 3.36 ± .054	0.504 ± 0.016 .397 ± .038
Fiber weight per boll (grams).....	A .87 ± .008 B .93 ± .016	.178 ± .006 .117 ± .011
Number of seeds per boll.....	A 17.6 ± .13 B 17.9 ± .22	2.853 ± .091 1.648 ± .016
Mean weight of individual seeds (grams).....	A .129 ± .0006 B .135 ± .0008	.0143 ± .0005 .0062 ± .0006
Lint index (grams).....	A 4.94 ± .029 B 5.18 ± .035	.635 ± .020 .258 ± .024

^a The standard deviations and probable errors of the means of series B have been corrected for the number (25).

⁷ A case of linkage between naked seeds and sparse lint and vice versa is reported by Thadani (15).

TABLE 3.—Coefficients of correlation in two series of samples of Pima cotton, 1925, these being (A) 224 individual bolls (B) 25 samples of 100 bolls each

Character pair	Coefficient of correlation	Character pair	Coefficient of correlation
Seed-cotton weight per boll and lint index.....	{ A .352±.039 B .590±.088	Fiber weight per boll and mean weight individual seeds.....	{ A -.104±.045 B .435±.109
Seed-cotton weight per boll and number seeds per boll.....	{ A .757±.019 B .934±.017	Lint index and number of seeds per boll.....	{ A -.073±.043 B .436±.109
Fiber weight per boll and lint index.....	{ A .542±.032 B .721±.065	Lint index and mean weight individual seeds.....	{ A .379±.038 B .387±.117
Fiber weight per boll and number of seeds per boll.....	{ A .700±.023 B .881±.030	Number of seeds per boll and mean weight individual seeds.....	{ A -.433±.036 B .396±.114

The standard deviation of every character (Table 2) was greater in Series A (224 individual bolls) than in Series B (25 samples of 100 bolls each) as would be expected since in the latter series the units are averages.

The correlations of seed cotton weight and of fiber weight per boll with lint index and with number of seeds per boll are in the same direction in both series, but in every case the correlation appears to be closer in Series B. For the correlation of lint index (mean weight of fiber per seed) with mean weight of the individual seed, the coefficients for the two series are nearly identical. The correlations of fiber weight per boll with mean weight of the individual seeds, lint index with number of seeds per boll, and number of seeds per boll with mean weight of the individual seeds gave coefficients in the two series which were of opposite sign and differed by an amount not less than 0.5.

As to the significance of the differences between the coefficients of correlation of the two series given in Table 3, it should be noted that the probable errors of the coefficients of correlation were computed by the usual formula $\left(\frac{0.6745 \times (1 - r^2)}{\sqrt{n}} \right)$. The number of samples in series B was only 25, and it has been shown recently by Fisher (3) that the probable errors of coefficients of correlation based on small numbers are too low when computed by this formula. Fisher's method of "transformed correlations" (3, p. 161-169) therefore has been used in determining the significance of the differences between the coefficients of series A and B. The differences as thus computed are shown in Table 4.

TABLE 4.—Significance of differences between the coefficients of correlation obtained on the two series of samples, A and B, as determined by the method of "transformed correlations"

Correlation	Difference between transformed correlations (z)	D/E
Seed-cotton weight per boll with lint index.....	0.31±.0.223	1.4
Seed-cotton weight per boll with number seeds per boll.....	.70±.223	3.1
Fiber weight per boll with lint index.....	.30±.223	1.3
Fiber weight per boll with number seeds per boll.....	.51±.223	2.3
Fiber weight per boll with mean weight of seeds.....	.57±.223	2.6
Lint index with number seeds per boll.....	.54±.223	2.4
Lint index with mean weight of seeds.....	.08±.223	0.1
Number seeds per boll with mean weight of seeds.....	.88±.223	3.9

Application of this method of transformed correlations brought out differences between the correlations in the two series of samples that were probably significant in only two cases, seed cotton weight with number of seeds per boll (D/E 3.1) and number of seeds per boll with mean weight of the individual seeds (D/E 3.9). The latter case is particularly interesting since the coefficients were of opposite sign and of nearly equal magnitude. In series A the coefficient of the partial correlation of number of seeds per boll with mean weight of the individual seeds *constant* for fiber weight per boll ($r -0.505 \pm 0.034$) does not differ significantly from that of the original correlation ($r -0.433 \pm 0.036$). In series B the original correlation ($r 0.396 \pm 0.114$) disappears in the partial correlation *constant* for fiber weight per boll ($r 0.030 \pm 0.135$). It is probable therefore that the apparently significant positive correlation in series B between the number and the mean weight of the seeds is conditioned by the very high positive correlation in this material between number of seeds and fiber weight per boll ($r 0.881 \pm 0.030$ as compared with 0.700 ± 0.023 in series A). The coefficients of the two series for the partial correlation of number of seeds per boll with mean weight of the individual seeds *constant* for fiber weight per boll do not differ significantly when compared as transformed correlations, the difference being only 0.43 ± 0.223 .

Conceivably, however, there might have been a real difference between the two series in the direction of the correlation between number of seeds per boll and mean weight of the individual seeds, if, in series B, in which the characters were positively correlated, both number and weight of the seeds had been affected in a like direction by the conditions, favorable or unfavorable, in the 25 fields in Salt River Valley from which the 25 samples were taken. The diversity of conditions among these fields was much greater than the diversity within the field at Sacaton in which the 224 bolls of series A were collected.⁸ A physiological tendency to decreasing weight of the individual seeds as their number in the boll increases may be assumed as the cause of the negative correlation in series A, the effect of which tendency, if present also in series B, may have been overcome by the greater influence of very diverse environmental conditions.

The data given in Table 3 shows clearly the danger of generalizing concerning the correlation of characters on the basis of a coefficient determined on a single population or under one set of conditions. The two populations here compared were of the same variety and were grown in the same year, yet for three pairs of characters they yielded coefficients of opposite sign. Analysis indicated that in this case the differences probably were not significant, but it is conceivable that significantly different coefficients of correlation might be given by subpopulations one of which had been subjected to a relatively uniform and the other to a very diverse environment.

⁸ Comparison of the standard deviations in Table 2 would suggest, on the contrary, that series A had been subjected to a greater diversity of environment than series B. But, as already mentioned, the units from which the statistical constants were computed were individual bolls in series A and averages of 100 bolls in series B. Hence comparison of the standard deviations of A and B is valueless as an indication of the relative diversity of the environments to which the two series had been exposed, for the standard deviations of series B represent merely the variation from field to field and do not take account of the variation within each of the 25 fields which, presumably, was of the order of magnitude indicated by the standard deviations of series A. If the 25 samples of 100 bolls each of series B could be regarded merely as multiplications of the one sample of 224 bolls constituting series A, the standard deviation of series B should have been only about one-tenth the observed standard deviation of series A. On this assumption, the standard deviations of series B for the several characters, as given in Table 2, are from 4 to 8 times the values that would have been obtained if the 2,500 bolls of series B had constituted a population as homogeneous as the 224 bolls of series A.

SYNOPSIS OF ALL CORRELATIONS DETERMINED IN ARIZONA

In addition to the two series of correlations determined in 1925 and discussed in the preceding sections, numerous correlations among the same and other boll, seed, and fiber characters had been determined in Arizona in previous years, chiefly on cottons of the Egyptian type but also on 180 F_2 plants of an upland-Egyptian hybrid grown at Sacaton in 1919 (8). It seems desirable therefore to bring together all available data on the correlation of each pair of characters, supplementing the Arizona data with references to correlations determined by other investigators, working with cottons of the upland type.⁹

The order in which the correlations are arranged is indicated by the following list of subjects:

- | | |
|-------------------------------------|----------------------|
| 1. Seed-cotton weight per boll. | 9. Fiber color. |
| 2. Fiber weight per boll. | 10. Seed fuzziness. |
| 3. Seed weight per boll. | 11. Boll length. |
| 4. Number of seeds per boll. | 12. Boll diameter. |
| 5. Mean weight of individual seeds. | 13. Boll index. |
| 6. Lint percentage. | 14. Boll apex index. |
| 7. Lint index. | 15. Boll volume. |
| 8. Fiber length. | |

In the case of the upland-Egyptian hybrid two different lots of material were used. The boll characters length, diameter, index, and apex index were determined on one unopen boll from each plant and the seed and fiber characters lint index, seed fuzziness, fiber length, and fiber color were determined on the contents of five matured bolls from each plant. Consequently only the correlations among the four boll characters and the correlations among the four seed and fiber characters will be considered in this section.

As to the units on which are based the correlations in Egyptian cottons, for characters 1 to 10 these were single bolls in only one series of samples, the 224 Pima bolls of 1925. In all other series, the units were the means of several bolls, each lot of bolls representing either a different individual plant or a different field. In a few cases the means of progenies, each progeny comprising several individual plants, were the units employed in computing the correlations. For characters 11 to 15 the units on which the correlations are based were in nearly all cases determinations made on single bolls (one per plant).

SEED-COTTON WEIGHT PER BOLL WITH FIBER WEIGHT PER BOLL.—The expected high and very significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.858 \pm 0.012$).

SEED-COTTON WEIGHT PER BOLL WITH NUMBER OF SEEDS PER BOLL.—High and very significant positive correlation was shown in 1925 by 224 individual bolls of Pima cotton ($r\ 0.757 \pm 0.019$) and by 25 samples of 100 bolls each of Pima cotton ($r\ 0.934 \pm 0.017$).

⁹ The most comprehensive data hitherto published on correlations of boll, seed, and fiber characters of cotton are those of:

Hodson (4), who determined correlations on from 34 to 85 individual plants of 2 different varieties in 1911 to 1917, on 48 varieties in 1917, and on 87 varieties in 1918. It may be inferred that in the last two series varietal means were the units in plotting the correlations.

Dunlavy (2), whose correlations were based on from 127 to 167 individual plants in Texas.

Martin and Mason (10), whose correlations were based on 811 individual plants in Nigeria.

Stroman (12), who determined correlations for each of 16 varieties in Texas, using 50 plants of each variety.

The units employed by the investigators cited in computing the correlations were not individual bolls but individual plants or varietal means.

SEED-COTTON WEIGHT PER BOLL WITH MEAN WEIGHT OF INDIVIDUAL SEEDS.—Not significantly correlated in 224 individual bolls of Pima cotton in 1925 ($r\ 0.105 \pm 0.045$). Dunlavy (2) found a high, positive, and very significant correlation ($r\ 0.664 \pm 0.034$) in upland cotton between what he terms "boll size" (weight of seed cotton per boll) and mean seed weight, and Hodson (4) in four populations of upland cottons obtained coefficients ranging from 0.506 ± 0.086 to 0.832 ± 0.030 .

SEED-COTTON WEIGHT PER BOLL WITH LINT PERCENTAGE.—A low but apparently significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.244 \pm 0.042$), while Dunlavy (2) records a significant negative correlation in upland cotton ($r - 0.394 \pm 0.051$). Hodson (4) determined this correlation in five populations of upland cotton and got significant coefficients in only two cases, one positive ($r\ 0.395 \pm 0.061$) and one negative ($r - 0.455 \pm 0.092$).

SEED-COTTON WEIGHT PER BOLL WITH LINT INDEX.—Positive and significant correlation was shown in 1925 by 224 individual bolls of Pima cotton ($r\ 0.352 \pm 0.039$) and by 25 samples of 100 bolls each of Pima cotton ($r\ 0.590 \pm 0.088$). Three other populations of Pima cotton in 1923 and 1924 gave coefficients of 0.502 ± 0.082 , 0.515 ± 0.068 and 0.588 ± 0.072 . Dunlavy (2) obtained in upland cotton a coefficient of 0.480 ± 0.046 .

SEED-COTTON WEIGHT PER BOLL WITH FIBER LENGTH.—This correlation was not determined in Arizona, but Hodson (4) reports for five samples of upland cottons coefficients ranging from 0.030 ± 0.115 to 0.300 ± 0.067 , and Dunlavy (2), who uses the term "boll size" for seed-cotton weight, got a coefficient for upland cotton of 0.214 ± 0.057 .

SEED-COTTON WEIGHT PER BOLL WITH PERCENTAGE 5-LOCK BOLLS PER PLANT.—As determined by Dunlavy (2) on upland cotton, a rather high positive correlation was shown ($r\ 0.533 \pm 0.058$).

SEED-COTTON WEIGHT PER BOLL WITH BOLL LENGTH.—A positive and very significant correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.418 \pm 0.037$).

SEED-COTTON WEIGHT PER BOLL WITH BOLL DIAMETER.—A positive, very significant, and fairly high correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.546 \pm 0.032$).

SEED-COTTON WEIGHT PER BOLL WITH BOLL INDEX.—Among the 224 individual bolls of Pima cotton in 1925 there was no correlation between weight of the matured contents of the boll and shape of the fully developed but unopen boll as indicated by its maximum diameter relative to its length ($r\ 0.008 \pm 0.045$).

FIBER WEIGHT PER BOLL WITH SEED WEIGHT PER BOLL.—As would be expected, a very high and very significant positive correlation was shown by 25 samples of 100 bolls each of Pima cotton in 1925 ($r\ 0.865 \pm 0.034$). The partial correlation constant for number of seeds gave a coefficient of only 0.250 ± 0.126 , indicating that the number of seeds in the boll rather than their individual weight determines the correlation with fiber weight.

FIBER WEIGHT PER BOLL WITH NUMBER OF SEEDS PER BOLL.—High and very significant positive correlation was shown in 1925 by 224 individual bolls of Pima cotton ($r\ 0.700 \pm 0.023$) and by 25 samples of 100 bolls each of Pima cotton ($r\ 0.881 \pm 0.030$). Three populations of Pima cotton in 1920 and 1923 gave coefficients of 0.48 ± 0.11 , 0.571 ± 0.034 and 0.628 ± 0.039 .

FIBER WEIGHT PER BOLL WITH MEAN WEIGHT OF INDIVIDUAL SEEDS.—No significant correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r - 0.104 \pm 0.045$), but 25 samples of 100 bolls each of Pima cotton in 1925 gave a coefficient of 0.435 ± 0.109 . It has been shown, however (Table 4) that owing to the small number of the latter series, these coefficients probably do not differ significantly. The apparent correlation shown by the 25 samples doubtless is due to the high correlation between fiber weight and number of seeds per boll ($r\ 0.88 \pm 0.03$) since the partial correlation of fiber weight per boll with mean weight of seeds constant for number of seeds per boll gave a coefficient of only 0.198 ± 0.129 .

FIBER WEIGHT PER BOLL WITH LINT PERCENTAGE.—A rather high and very significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.602 \pm 0.029$).

FIBER WEIGHT PER BOLL WITH LINT INDEX.—Fairly high and very significant positive correlation was shown in 1925 by 224 individual bolls of Pima cotton ($r\ 0.542 \pm 0.032$), and a still higher correlation by 25 samples of 100 bolls each of Pima cotton ($r\ 0.721 \pm 0.065$). The partial correlation constant for number of seeds per boll increases the coefficient of the first series to 0.830 ± 0.014 and

that of the second series to 0.790 ± 0.051 . Twelve samples of Pima cotton from as many fields in Salt River Valley in 1920 gave a correlation between fiber weight per boll and lint index of 0.90 ± 0.03 .

FIBER WEIGHT PER BOLL WITH BOLL LENGTH.—The correlation shown by 224 individual bolls of Pima cotton in 1925 ($r 0.415 \pm 0.037$) was almost identical with the correlation in the same material between seed-cotton weight and boll length.

FIBER WEIGHT PER BOLL WITH BOLL DIAMETER.—The correlation shown by 224 individual bolls of Pima in 1925 ($r 0.445 \pm 0.036$) was lower but not significantly lower than the correlation between seed-cotton weight and boll diameter.

FIBER WEIGHT PER BOLL WITH BOLL INDEX.—As in the case of seed-cotton weight per boll with boll index, no significant correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r 0.093 \pm 0.045$).

The correlation of fiber weight per boll with seed-cotton weight per boll has been mentioned under the latter character as subject.

SEED WEIGHT PER BOLL WITH NUMBER OF SEEDS PER BOLL.—As would be expected, these characters are very closely correlated, the coefficient for 25 samples of 100 bolls each of Pima cotton in 1925 having been 0.934 ± 0.017 .

SEED WEIGHT PER BOLL WITH LINT PERCENTAGE.—Two series of samples of Egyptian cotton grown in Arizona in 1910 (5) showed the expected negative correlation ($r -0.63 \pm 0.047$ and -0.40 ± 0.092).

The correlation of seed weight per boll with fiber weight per boll has been mentioned under the latter character as subject.

NUMBER OF SEEDS PER BOLL WITH MEAN WEIGHT OF INDIVIDUAL SEEDS.—A fairly high and very significant negative correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r -0.433 \pm 0.036$), while a positive correlation of nearly equal magnitude was shown by 25 samples of 100 bolls each of Pima cotton in 1925 ($r 0.396 \pm 0.114$). As was suggested on a preceding page, this difference probably is not significant.

NUMBER OF SEEDS PER BOLL WITH LINT PERCENTAGE.—A rather low but significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r 0.284 \pm 0.041$).

NUMBER OF SEEDS PER BOLL WITH LINT INDEX.—These characters were not significantly correlated in 224 individual bolls of Pima cotton in 1925 ($r -0.073 \pm 0.045$), but an apparently significant positive correlation was shown by 25 samples of 100 bolls each of Pima in 1925 ($r 0.436 \pm 0.109$). It has been shown, however (Table 4), that when allowance is made for the small number in the second series, the difference between the two coefficients is not significant. In the 25 samples, the apparent correlation probably was due to the high positive correlation between fiber weight and number of seeds per boll ($r 0.88 \pm 0.03$), since the partial correlation of number of seeds per boll with lint index, *constant* for fiber weight per boll, gave a very significant negative coefficient ($r -0.607 \pm 0.085$). The same partial correlation for the 224 individual bolls gave a coefficient of -0.750 ± 0.020 .

NUMBER OF SEEDS PER BOLL WITH BOLL LENGTH.—A rather low but quite significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r 0.310 \pm 0.041$).

NUMBER OF SEEDS PER BOLL WITH BOLL DIAMETER.—A very significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r 0.380 \pm 0.038$).

NUMBER OF SEEDS PER BOLL WITH BOLL INDEX.—There was no correlation between number of seeds and shape of the boll, as represented by its maximum diameter relative to its length (boll index) in 224 individual bolls of Pima cotton in 1925 ($r -0.041 \pm 0.045$).

The correlations of number of seeds per boll with seed-cotton weight per boll, fiber weight per boll, and seed weight per boll have been mentioned under those characters as subjects.

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH LINT PERCENTAGE.—A fairly high and very significant negative correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r -0.432 \pm 0.036$). In upland cotton Dunlavy (2) found a very significant negative correlation ($r -0.529 \pm 0.038$), as did also Martin and Mason (10) who obtained a coefficient of -0.34 ± 0.021 . Hodson (4) found a significant correlation ($r -0.40 \pm 0.08$) in only one of four populations of upland cottons.

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH LINT INDEX.—A fairly high and very significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r 0.379 \pm 0.038$) and an almost identical degree of correlation

by 25 samples of 100 bolls each of Pima cotton in 1925 ($r\ 0.357 \pm 0.117$). A much closer association of weight of the seed and weight of the fiber borne on it (lint index) is reported by Dunlavy (2) for the upland cotton with which he worked ($r\ 0.704 \pm 0.021$) and by Martin and Mason (10) who also worked with upland cottons ($r\ 0.56 \pm 0.016$). Patel (11) in three strains of *Gossypium herbaceum* in India obtained coefficients of from 0.46 ± 0.05 to 0.73 ± 0.03 .

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH FIBER LENGTH.—This correlation was not determined in Arizona, but Hodson (4) reports for five populations of upland cottons coefficients ranging from -0.04 ± 0.11 to 0.30 ± 0.066 and 0.33 ± 0.10 . Dunlavy (2), also working with upland cottons, got a coefficient of 0.426 ± 0.043 .

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH PERCENTAGE 5-LOCK BOLLS PER PLANT.—Dunlavy (2) found no significant correlation in upland cotton ($r\ 0.114 \pm 0.055$).

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH BOLL LENGTH.—No correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.059 \pm 0.045$).

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH BOLL DIAMETER.—A low but apparently significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.189 \pm 0.043$).

MEAN WEIGHT OF INDIVIDUAL SEEDS WITH BOLL INDEX.—No correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.076 \pm 0.045$).

The correlations of the mean weight of individual seeds with seed cotton weight per boll, fiber weight per boll, and number of seeds per boll have been mentioned under those characters as subjects.

LINT PERCENTAGE WITH LINT INDEX.—A fairly high and very significant positive correlation was shown in 1925 by 224 individual bolls of Pima cotton ($r\ 0.540 \pm 0.032$). Closer correlation was shown (5) in two populations of Egyptian cotton grown in Arizona in 1910 ($r\ 0.64 \pm 0.045$ and 0.33 ± 0.034) and in a population of Pima cotton in 1924 ($r\ 0.868 \pm 0.023$). Martin and Mason (10) obtained a coefficient of 0.42 ± 0.02 and Dunlavy (2) the exceptionally low coefficient of 0.203 ± 0.050 .

LINT PERCENTAGE WITH FIBER LENGTH.—The correlation of these characters was not determined on Arizona material. Other investigators report coefficients for upland cotton as follows: Hodson (4) in five populations obtained coefficients ranging from -0.034 to -0.311 , three of which (all negative) may have been significant; Dunlavy (2) -0.445 ± 0.042 ; Martin and Mason (10) -0.21 ± 0.023 ; and Stroman (12) significant correlations in only 4 out of 16 varieties, all negative, with coefficients ranging from -0.37 ± 0.09 to -0.52 ± 0.07 . Negative correlation between lint percentage and fiber length in upland cotton is strongly indicated by these data. Kottur reports these characters to be independent in the Indian cottons with which he worked (9, p. 129-133).

LINT PERCENTAGE WITH PERCENTAGE 5-LOCK BOLLS PER PLANT.—Dunlavy (2) found no significant correlation in upland cotton ($r\ 0.036 \pm 0.056$).

LINT PERCENTAGE WITH BOLL LENGTH.—A low but apparently significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.179 \pm 0.044$).

LINT PERCENTAGE WITH BOLL DIAMETER.—No correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.074 \pm 0.045$).

LINT PERCENTAGE WITH BOLL INDEX.—A low but possibly significant negative correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ -0.142 \pm 0.044$). It has been shown, however, that this apparent correlation probably is due to the high negative correlation of boll length with boll index.

The correlations of lint percentage with seed cotton weight per boll, fiber weight per boll, seed weight per boll, number of seeds per boll, and mean weight of individual seeds have been mentioned under those characters as subjects.

LINT INDEX WITH FIBER LENGTH.—This correlation was determined in Arizona on seven populations of Egyptian cotton, of which six were of the Pima variety. In only three populations (all of Pima cotton) the coefficients were apparently significant, having been 0.360 ± 0.055 , -0.132 ± 0.038 , and -0.361 ± 0.085 . Since one of the apparently significant coefficients was positive and the other two negative, no general conclusion in regard to this correlation is possible. For 180 F_2 plants of an upland-Egyptian hybrid grown at Sacaton in 1919 the coefficient of correlation was -0.098 ± 0.050 . Dunlavy (2) reports a coefficient of correlation between fiber length and lint index of 0.153 ± 0.051 , while Martin and Mason (10) obtained a coefficient of only 0.07 ± 0.024 .

LINT INDEX WITH FIBER COLOR.—No correlation was shown by 180 F_2 plants of an upland-Egyptian hybrid at Sacaton in 1919 ($r\ 0.033 \pm 0.050$).

LINT INDEX WITH SEED FUZZINESS.—No correlation was shown by 180 F_2 plants of an upland-Egyptian hybrid in 1919 ($r\ 0.090 \pm 0.050$), by 178 individual selections of Pima cotton in 1922 ($r\ -0.061 \pm 0.050$), and by 224 individual bolls of Pima cotton in 1925 ($r\ 0.042 \pm 0.045$). A coefficient of -0.327 ± 0.100 was obtained on 36 individual selections of Sakellaridis cotton at Sacaton in 1922, but the population was too small to make it likely that this correlation is significant.

LINT INDEX WITH PERCENTAGE 5-LOCK BOLLS PER PLANT.—Dunlavy (2) found no significant correlation in upland cotton ($r\ 0.078 \pm 0.056$). In the second generation population of an upland-Egyptian hybrid grown at Sacaton in 1919 (180 plants) there was no correlation between lint index and mean lock number per plant ($r\ 0.014 \pm 0.050$).

LINT INDEX WITH BOLL LENGTH.—A low but significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.237 \pm 0.042$).

LINT INDEX WITH BOLL DIAMETER.—A low but significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.228 \pm 0.042$).

LINT INDEX WITH BOLL INDEX.—No significant correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ -0.100 \pm 0.045$).

The correlations of lint index with seed cotton weight per boll, fiber weight per boll, number of seeds per boll, mean weight of individual seeds, and lint percentage have been mentioned under those characters as subjects.

FIBER LENGTH WITH FIBER COLOR.—For 180 F_2 plants of an upland-Egyptian hybrid grown in Arizona in 1919 the coefficient of correlation was -0.230 ± 0.048 indicating a slight but probably significant tendency for the plants with longer fiber to have lighter colored fiber. Kottur (3, p. 124-129) noted a tendency in Indian cottons for shortness of staple to be associated with brown color of the fiber, but he did not determine the coefficient of correlation.

FIBER LENGTH WITH SEED FUZZINESS.—No significant correlation was shown in Arizona by three populations, one of the Pima variety ($r\ 0.046 \pm 0.051$) one of the Sakellaridis variety ($r\ 0.283 \pm 0.103$), and one of the second generation of an upland-Egyptian hybrid ($r\ 0.050 \pm 0.050$).

FIBER LENGTH WITH PERCENTAGE 5-LOCK BOLLS PER PLANT.—Dunlavy (2) found no significant correlation in upland cotton ($r\ -0.109 \pm 0.055$). In the second generation of an upland-Egyptian hybrid at Sacaton in 1919 (180 plants) fiber length and boll lock number (mean per plant) were not significantly correlated ($r\ -0.127 \pm 0.049$).

The correlations of fiber length with seed cotton weight per boll, mean weight of individual seeds, lint percentage, and lint index have been mentioned under those characters as subjects.

FIBER COLOR WITH SEED FUZZINESS.—For 180 plants of upland-Egyptian F_2 in Arizona in 1919 the coefficient of correlation was negligible ($r\ -0.016 \pm 0.050$).

The correlations of fiber color with lint index and fiber length have been mentioned under those characters as subjects.

The correlations of seed fuzziness with lint index, fiber length, and fiber color have been mentioned under those characters as subjects.

BOLL LENGTH WITH BOLL DIAMETER.—A fairly high and very significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ 0.385 \pm 0.038$). This correlation was determined in Arizona also on seven other populations of Pima and other varieties of Egyptian cotton. The coefficients ranged from 0.21 to 0.73 (0.5 or higher in six cases), and were significant in all but one case. For 180 F_2 plants (one boll per plant) of an upland-Egyptian hybrid at Sacaton in 1919 the coefficient of correlation was 0.289 ± 0.046 .

BOLL LENGTH WITH BOLL INDEX.—The expected high and very significant negative correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r\ -0.680 \pm 0.023$). As determined in Arizona on seven other populations representing several Egyptian varieties, the coefficients of correlation ranged from -0.59 ± 0.07 to -0.795 ± 0.028 . For 180 F_2 plants of an upland-Egyptian hybrid grown in 1919 the coefficient was -0.703 ± 0.025 .

BOLL LENGTH WITH BOLL APEX INDEX.¹⁰—For 180 F_2 plants of an upland-Egyptian hybrid grown at Sacaton in 1919 the coefficient of correlation was -0.504 ± 0.038 .

BOLL LENGTH WITH BOLL VOLUME.—The correlation with volume of the fully developed but unopen boll (as measured by the displacement of water) was determined in 1916 on three lots of bolls, each boll having been from a different

¹⁰ The boll apex index is the diameter 5 mm below the apex taken as a percentage of the maximum diameter. Round bolls have a high, and pointed bolls a low, index.

individual plant. For 161 bolls of the Pima variety the coefficient was 0.64 ± 0.031 ; for 207 bolls of the Gila variety, 0.73 ± 0.022 ; and for 407 bolls of Pima \times Gila F_2 , 0.62 ± 0.021 . The length of the boll therefore gives a good indication of its volume.

The correlations of boll length with seed cotton weight per boll, fiber weight per boll, number of seeds per boll, mean weight of individual seeds, lint percentage, and lint index are mentioned under those characters as subjects.

BOLL DIAMETER WITH BOLL INDEX.—A significant positive correlation was shown by 224 individual bolls of Pima cotton in 1925 ($r 0.325 \pm 0.040$). Of seven other populations of Egyptian cotton in Arizona only three gave significant coefficients ($r 0.116 \pm 0.032$, 0.23 ± 0.05 , and 0.467 ± 0.069). The coefficient for 180 F_2 plants of an upland Egyptian hybrid was 0.376 ± 0.043 .

BOLL DIAMETER WITH BOLL APEX INDEX.—For 180 F_2 plants of an upland Egyptian hybrid grown at Sacaton in 1919 the coefficient of correlation was -0.079 ± 0.050 .

BOLL DIAMETER WITH BOLL VOLUME.—This correlation was determined on the same populations as was the correlation of boll length with boll volume, and the coefficients obtained were: Pima, 0.73 ± 0.025 ; Gila, 0.82 ± 0.015 ; and Pima \times Gila F_2 , 0.79 ± 0.012 . The volume of the boll therefore was even better indicated by the maximum external diameter than by the length. It is possible also that diameter is more closely correlated than length with the weight of seed cotton and of fiber per boll. (Table 1.)

The correlations of boll diameter with seed-cotton weight per boll, fiber weight per boll, number of seeds per boll, mean weight of individual seeds, lint percentage, lint index, and boll length are mentioned under those characters as subjects.

BOLL INDEX WITH BOLL APEX INDEX.—For 180 F_2 plants of an upland Egyptian hybrid grown at Sacaton in 1919 the correlation was significant and positive ($r 0.448 \pm 0.040$), as would be expected since relatively slender bolls usually have pointed tips, and vice versa.

The correlation of boll index with other characters are mentioned under those characters as subjects.

The correlations of boll apex index with boll length, boll diameter, and boll index are mentioned under those characters as subjects.

The correlations of boll volume with boll length and boll diameter are mentioned under those characters as subjects.

EVIDENCE OF GENETIC CORRELATION

All of the correlations discussed in this paper which gave high coefficients are more or less obviously physical or physiological. The populations on which the correlations were determined were not of a nature to afford evidence as to the occurrence of genetic correlation, except in the case of 180 F_2 plants of an upland-Egyptian hybrid, grown in 1919 (8). In this population the coefficients of correlation were computed for all possible combinations of the following characters:

- | | |
|----------------------|---------------------|
| 1. Lint index. | 6. Boll length. |
| 2. Fiber length. | 7. Boll diameter. |
| 3. Fiber color. | 8. Boll index. |
| 4. Seed fuzziness. | 9. Boll apex index. |
| 5. Boll-lock number. | |

The first four characters were determined as the average for five mature bolls from each plant and the last four characters on a single, fully developed but unopen boll on each plant. The mean boll-lock number for each plant was computed by counting the number of locks in every boll on the plant. The coefficients of correlation were computed by the product-moment method, none of the characters having shown segregation in definite ratios.¹¹

¹¹ The seed coat character (seed fuzziness) in crosses between cottons which have, respectively, naked and fuzzy seeds, shows definite monohybrid segregation, absence of fuzz being dominant. But such segregation was not observed in the upland Egyptian hybrid under consideration, both parents of the hybrid having had more or less fuzzy seeds.

Ten of the 36 pairs of characters gave coefficients of correlation amounting to three or more times the probable error. These coefficients are given in Table 5.

TABLE 5.—*Significant correlations among seed, fiber, and boll characters of an upland × Egyptian F₂ population (180 plants)*

Character pair	Coefficient of correlation (r)	r/E	Character pair	Coefficient of correlation (r)	r/E
Lint index.....	0.214±0.048	4.5	Boll length.....	0.289±0.046	6.3
Boll diameter.....			Boll diameter.....		
Fiber length.....	.175±.049	3.6	Boll length.....	-.703±.025	28.1
Boll length.....			Boll index.....		
Fiber length.....	-.172±.049	3.5	Boll length.....	-.504±.038	13.3
Boll apex index.....			Boll apex index.....		
Fiber length.....	-.230±.048	4.8	Boll diameter.....	.376±.043	8.7
Fiber color.....			Boll index.....		
Boll lock number.....	.202±.048	4.2	Boll index.....	.448±.040	11.2
Boll diameter.....			Boll apex index.....		

All but the first four correlations in Table 5 may be dismissed as obviously or probably of a physical or physiological nature. That this applies to the intercorrelations of boll length, boll diameter, and boll index (diameter as a percentage of the length) has been brought out in preceding pages. The positive correlation between boll lock number and boll diameter is obviously physical. A much closer correlation doubtless would be shown by determinations on the same individual bolls. The negative correlation between boll length and boll apex index (diameter 5 mm. below the apex as a percentage of the maximum diameter) is probably a physiological one, since the most cursory observation of a series of cotton varieties shows that long bolls tend to be pointed, and vice versa. A similar relation is expressed in the positive correlation of boll index with boll apex index which indicates that slender bolls (having a low boll index) tend to be pointed and vice versa.

The positive correlation of lint index with boll diameter, although coherent (in the direction indicated by the parental associations of the characters) is in all probability a physiological one, since the 224 individual bolls of Pima cotton in 1925 gave very nearly the same coefficient ($r = 0.228 \pm 0.042$). The correlation probably expresses merely a like influence of environmental conditions upon two otherwise independent characters.

The low positive correlation of fiber length with boll length and the low negative correlation of fiber length with boll apex index probably indicate a slight physiological tendency for the longer and more pointed bolls to contain longer fiber. A comparison of different types and varieties of cotton shows that the long-staple varieties mostly have long and pointed bolls. Both correlations are coherent, however, and the evidence at hand does not exclude the possibility that a weak linkage is involved. Closer correlation might have been shown if the fiber character and the boll characters had been determined on the same individual bolls. The correlations of the two boll characters with fiber length evidently are not independent, being connected by the rather high negative correlation between boll length and boll apex index ($r = -0.504 \pm 0.038$). This is shown by

the partial correlations, that of fiber length with boll length *constant* for boll apex index giving a coefficient of only 0.103 ± 0.050 and that of fiber length with boll apex index *constant* for boll length giving a coefficient of only 0.098 ± 0.050 .

The only one of these ten correlations in which physical or physiological association of the characters seems improbable is the negative correlation between fiber length and fiber color, the coefficient ($r = 0.230 \pm 0.048$) being nearly five times its probable error. This correlation is, however, disherent, the upland parent having had shorter and lighter colored fiber than the Egyptian parent, whereas the hybrid shows a tendency for longer fiber to be associated with lighter colored fiber and vice versa. Such a correlation, if it is really genetic, may be interpreted only on the unproven assumption that crossing-over in excess of 50 per cent has occurred. An association of short staple with brown color in Indian cottons was noticed by Kottur (9, p. 124-129), but he did not determine the coefficient of correlation.

The only case in which linkage of any of the characters of the cotton plant discussed in this paper appears to have been demonstrated is that between sparse lint (low lint index) and naked seeds, reported by Thadani (13).

SUMMARY

The intercorrelations of various characters of the bolls, seeds, and fiber have been determined in Arizona on several populations of cottons of the Egyptian type, and on a population of 180 second generation plants of a hybrid between upland and Egyptian cotton.

The coefficients of correlation were computed on 224 (or 250) individual bolls borne by 50 plants of Pima Egyptian cotton at Sacaton, Ariz., in 1925, for all possible combinations of the characters seed-cotton weight per boll, fiber weight per boll, lint percentage, lint index, number of seeds per boll, mean weight of the individual seeds, boll length, boll diameter, and boll index, a total of 36 pairs of characters. The coefficients of correlation are given in Table 1. For 26 pairs of characters the coefficients are probably significant, being more than three times the probable error.

There were also computed in 1925 coefficients of correlation for eight pairs of characters on 25 samples from as many fields in the Salt River Valley, Ariz., each sample having consisted of 100 bolls. A comparison of these coefficients with the coefficients obtained for the same pairs of characters on the 224 individual bolls is made in Tables 3 and 4. For three of the eight pairs of characters the coefficients differ markedly, although the significance of the differences is doubtful. This comparison shows clearly, however, the danger of generalizing as to the correlation of characters on the basis of a coefficient determined on a single population and under one set of environmental conditions.

These and all other correlations determined in Arizona, together with references to the published data of other investigators who worked chiefly with upland cottons, are brought together in a synopsis. The subjects of the correlations are arranged so as to facilitate reference.

Evidence as to the occurrence of linkage, afforded by the correlations of characters in a second-generation population of an upland-Egyptian hybrid, is discussed in the concluding section. Coefficients

higher than 0.25 were obtained in the hybrid only in cases where the correlation is obviously or very probably of a physical or physiological nature, in other words due to the mathematical relationship of the characters or to a like effect of environmental conditions upon otherwise independent characters.

Among the characters considered in this paper, the only significant correlation found by the writer which appears to be neither physical nor physiological is the negative correlation between fiber length and fiber color in the upland-Egyptian hybrid, which gave a coefficient of -0.230 ± 0.048 . In this case the correlation is disherent, since in the parents of the hybrid long fiber was associated with deeper colored fiber, and vice versa. Linkage in this case would have been indicated by a positive correlation unless crossing over in excess of 50 per cent is assumed to have taken place.

An apparently well-substantiated case of linkage between sparseness of the fiber and absence of fuzz on the seeds and vice versa, has been reported by Thadani (13).

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NOTES ON SOME LEPIDOPTERA FROM EASTERN TEXAS¹

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INTRODUCTION

In the latter part of 1922, and again in 1923, the writer was detailed to collect and rear Lepidoptera from eastern Texas, especially those of the Malvaceae. The project was carried out in connection with work on the eradication of the pink bollworm (*Pectinophora gossypiella* Saunders), under the supervision of the late W. D. Hunter, in charge. The main objects of the work were twofold; to discover, if possible, host plants of the pink bollworm other than cotton, and to enlarge the list of Lepidoptera likely to be mistaken for this comparatively new cotton pest.

Most of the collections were made in Liberty and Chambers Counties, in parts of which infestations of the pink bollworm had been found in 1917, 1919, and 1920. In 1918 and 1919 a similar study of the lepidopterous insects of malvaceous and related plants was made and the results published.³ The pink bollworm was not found on any of the plants examined.

Of the 40 species of Lepidoptera listed in the present paper, four are described as new, and two, already described, are here recorded for the first time from the United States. One variety is also described as new, and one new genus has been erected by Busck. In the course of the work a large number of observations were made on rather well-known species. The present paper, however, has been confined practically altogether to new species and to previously unrecorded data regarding the habits and distribution of other forms.

FAMILY HESPERIIDAE

PYRGUS SYRICHTUS (FABRICIUS)

Papilio syrichtus Fabricius, 1775, Syst. Ent., p. 534.

Hesperia syrichtus Dyar, 1902, List N. Amer. Lepidop., no. 652.

Pyrgus syrichtus Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 493, 1917.

Larvae of this species were collected on leaves of the following malvaceous plants during 1923: *Sida rhombifolia* Linnaeus, at

¹ Received for publication May 12, 1926; issued November, 1926.

² The writer is indebted to Schaus, Busck, and Heinrich for determining the Lepidoptera and for other assistance; to Rohwer, Gahan, and Cushman for naming the Hymenoptera; and to Aldrich for identifying the dipterous parasites. He is also indebted to Bessey, of East Lansing, Mich., and Standley, of the National Herbarium, for determining the host plants. The drawings were made by H. B. Bradford, under the supervision of August Busck.

³ HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 807-836, illus. 1921.

Liberty, Tex., July 7; *Malvastrum americanum* (Linnaeus) Torrey, at Victoria, Tex., August 2 and August 25; and on the same host plants at Lake Charlotte, Tex., November 16. An adult emerged July 24, 1923, from a specimen taken at Liberty.

FAMILY SATURNIIDAE

AUTOMERIS IO (FABRICIUS)

Bombyx io Fabricius, 1775, Syst. Ent., p. 560.

Automeris io Dyar, 1902, List N. Amer. Lepidop. no. 753; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 780.

A colony of about 25 half-grown larvae of this moth was found defoliating a cotton plant at Liberty August 31, 1923. The larvae collected became mature during the last week of the month following and pupated within tough, oval-shaped, brownish cocoons. From these specimens several adults were reared during the latter half of April, 1924.

A single immature larva was also taken on cotton at Smith Point, Tex., October 4, 1923. Several days later two young larvae were collected on leaves of *Hibiscus lasiocarpus* Cavanilles at Liberty. A nearly full-grown larva was found feeding upon the leaflets of *Amorpha fruticosa* Linnaeus at Lake Charlotte, October 23, 1923.

FAMILY NOCTUIDAE

SUBFAMILY AGROTINAE

HELIOTHIS OBSOLETA (FABRICIUS)

Bombyx obsoleta Fabricius, 1793, Ent. Syst., 3 (1): 456.

Chloridea obsoleta Hampson, 1903, Cat. Lepidop. Phal. Brit. Mus. 4: 45, 657.

Heliothis obsoleta Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 1090.

Larvae of this species, which is known as the "cotton bollworm," were collected in maturing bolls of *Hibiscus lasiocarpus* Cavanilles and *H. militaris* Cavanilles at Lake Charlotte from August 18 to September 8, 1922, and in the bolls and open flowers of *H. lasiocarpus* at Liberty during September of the same year. Pupation took place within oval-shaped cells in the soil, the average pupation period of the five adults reared being 11 days. The adults emerged from September 11 to October 13, 1923.

Larvae were also found feeding upon leaves and bolls of okra (*Hibiscus esculentus* Linnaeus) at Smith Point October 3, 1923. One adult emerged October 30, 1923, and two emerged April 8 and April 17, 1924.

HELIOTHIS VIRESCENS (FABRICIUS)

Noctua virescens Fabricius, 1781, Spec. Ins. 2: 216.

Chloridea virescens Dyar, 1902, List N. Amer. Lepidop., no. 2296.

Heliothis virescens Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 1091.

Larvae were collected on leaves and bolls of okra (*Hibiscus esculentus* Linnaeus) at Smith Point October 3, 1923. The larvae pupated the same month, and from these specimens three adults were reared during the first part of the following April.

SUBFAMILY ERASTRIINAE

XANTHOPTERA NIGROFIMBRIA GUENÉE

Xanthoptera nigrofimbria Guenée, 1852, Spec. Gén. 6: 241.

One larva was found in a ripening capsule of *Ipomoea speciosa* Walter at Smith Point, September 1, 1922. Pupation took place about September 3 within a thin cocoon fastened to the stem of the plant and covered with small pieces of leaf. The moth emerged September 15, 1922.

HELIOCONTIA MARGANA (FABRICIUS)

Paralis margana Fabricius, 1794, Ent. Syst., 3 (2): 257.

Spragueia sordida Dyar, 1902, List N. Amer. Lepidop., no. 2710.

Heliocontia margana Hampson, 1910, Cat. Lepidop. Phal. Brit. Mus. 10: 666-667; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 2924.

Larvae of this species were collected on the leaves and buds of *Malvastrum americanum* (Linnaeus) Torrey at Victoria August 21, 1923. From this material a male emerged September 3 of the same year.

SUBFAMILY ACONTIINAE

BAGISARA RECTIFASCIA (GROTE)

Schinia rectifascia Grote, 1874, Boston Soc. Nat. Hist. Proc. 16: 242.

Alethmia rectifascia Dyar, 1902, List N. Amer. Lepidop., no. 2267.

Bagisara rectifascia Hampson, 1910, Cat. Lepidop. Phal. Brit. Mus. 9: 156; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 2730; Heinrich, 1921, Jour. Agr. Research 20: 834.

Larvae were collected on leaves of *Hibiscus lasiocarpus* (?) at Smith Point August 10, 1922. One specimen pupated August 13 in a loosely folded leaf, and the adult emerged 11 days later. An adult was reared November 7, 1923, from larvae collected on leaves of *Malvariscus drummondii* Torrey and Gray at the same locality October 3, 1923.

The larvae were found in large numbers on *Malvaviscus* at Lake Charlotte during October, 1923. When full grown the larva enters the soil and constructs a flattened circular cell which is lined with silk, and in which it rests until it pupates the following spring. No adults were obtained from these larvae.

The full-grown larva is 30 to 35 mm. long, 2 to 2½ mm. wide, and nearly cylindrical. The body above is light green, with seven narrow, wavy stripes of darker green, one dorsal, two subdorsal on each side, and one lateral; paler beneath. Head pale greenish white with four transverse broken rows of grayish spots; thoracic shield and anal plate of body color; thoracic legs pale; abdominal legs pale, rather elongate, one pair each on fifth and sixth abdominal segments; (absent on third and fourth abdominal segments); crochets about 20 in number, arranged in a semicircle on inner side; anal legs pale, well developed; anal fork lacking; body setae elongate and slender. The dorsal surface of the full-grown larva is often tinged with a pale wine color.

Heinrich has advised the writer that this species should be placed in the Acontiinae rather than in the Acronyctinae because of characters of the larva and the adult, the latter having vein 5 of the hind wing well developed.

Larvae collected at Lake Charlotte were found to be parasitized by *Meteorus laphygmae* Viereck (Hymenoptera). Two of these emerged about December 1, 1923, from oval-shaped cocoons spun by the parasitic larvae after the latter had left the body of the host.

SUBFAMILY PHYTOMETRINAE

PHYTOMETRA OO (CRAMER)

[*Phalaena*] oo Cramer, 1782, Pap. Exot. 4: 45, pl. 311, fig. E (nec. F).
Phytometra oo Hampson, 1913, Cat. Lepidop. Phal. Brit. Mus. 13: 522.

A single adult was reared about December 1, 1923, from a larva found in a fold of an okra leaf at Smith Point November 1, 1923. The larva was a very pale green, except for a large blackish area on the middle of the dorsal surface. The dark area was present on the pupa and is retained on the pupal case.

SUBFAMILY EREBINAE

ANOMIS EROSA HÜBNER

Anomis erosa Hübner, 1818, Zutr. Samml. Exot. Schmett., p. 19, fig. 287, 288; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 3407.

Larvae and pupae were collected in large numbers on okra at Smith Point during October, 1923. The larvae feed upon the leaves and pupate in a long roll or fold in the leaf. Emergence dates ranged from October 8 to about December 1, 1923.

From this material four adults of the hymenopterous parasite *Itopectis conquisitor* (Say) were reared during December, 1923, each parasite developing singly within the body of its host and emerging during the pupal stage of the latter.

One larva of *Anomis erosa* was taken on *Malvaviscus drummondii* Torrey and Gray at Lake Charlotte October 23, 1923. The specimen pupated one week later in a loosely tied leaf, and about December 1 of the same year a dipteran, *Zenillia blanda* Osten Sacken, emerged from the pupa.

A series of adults of the hymenopteron *Syntomosphyrum esurus* Riley emerged from a pupa of *Anomis erosa* during the first part of December, 1923. A single emergence hole in the dorsal surface of the abdomen of the host was made. This parasite was also reared in large numbers during the winter of 1923-24 from pupae of *Alabama argillacea* (Hübner), taken on cotton in various parts of Liberty and Chambers Counties.

ANOMIS EXACTA HÜBNER

Anomis exacta Hübner, 1810, Samml. Exot. Schmett., v. 2, pl. 411; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 3410.

One larva was collected on *Malvaviscus* at Lake Charlotte October 12, 1923. The specimen pupated October 17 in a loosely tied leaf of the host plant, and the adult emerged November 8, 1923.

FAMILY GEOMETRIDAE

SYNCHLORA DENTICULARIA (WALKER)

Nemoria (?) *denticularia* Walker, 1861, List Lepidop. Brit. Mus. 22: 536.

Synchlora excurraria Hulst, 1895, Ent. News 6: 71.

Synchlora denticulata Dyar, 1902, List N. Amer. Lepidop., no. 3580.

Synchlora denticularia Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 3801.

Four adults were reared June 30, to July 16, 1923, from pupae collected on the flower heads of *Rudbeckia maxima* Nuttall at Liberty June 27 and July 7, 1923. One larva was taken on the flower head of *Helianthus* sp. at Smith Point October 2, and a second on *Malvastrum americanum* (Linnaeus) Torrey at Lake Charlotte November 16, 1923. An adult was reared October 20, 1923, from the first specimen. The larvae are surface feeders, and carry about with them seeds and particles of the host plant attached to the spiny tubercles on the dorsal surface of the abdominal segments. Pupation takes place within a very frail web covered with small pieces of the host plant.

FAMILY PYRALIDAE

SUBFAMILY PYRAUSTINAE

LOXOSTEGE HELVIALIS (WALKER)

Spilodes helvialis Walker, 1859, List Lepidop. Brit. Mus. 18: 772.

Loxostege helvialis Dyar, 1902, List N. Amer. Lepidop., no. 4351; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5022.

Several larvae of this species were found feeding within loosely rolled leaves of *Amaranthus spinosus* Linnaeus, at Liberty July 2 to 4, 1923. On July 4 one larva was also taken in a rolled leaf of *Sida rhombifolia* Linnaeus, in close proximity to some of the *Amaranthus* plants. Feeding of the larva on *Sida* was not noted.

The feeding larva is pale green, and when full grown is about 18 mm. in length. Just before pupation its dorsal surface becomes either a solid deep pink or is marked with broad stripes of dark pink. Moths emerged July 11 and July 16, 1923, from the tough, dark brown cocoons, to the outside of which leaves of the host plant had been fastened. From a whitish cocoon spun by one of the larvae a dipteran emerged July 18, but it was in poor condition and could not be determined.

PYRAUSTA PHOENICEALIS (HÜBNER)

Haematia phoenicealis Hübner, 1818, Zutr. Exot. Schmett., fig. 115, 116.

Pyrausta phoenicealis Dyar, 1902, List N. Amer. Lepidop., no. 4448; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5145.

Larvae of this species were collected in frail silken tubes in the flower heads of a mint, *Mesosphaerum rugosum* (Linnaeus) Pollard, at Liberty, during September and at Smith Point on October 2, 1923. The full-grown larva is about 15 mm. long, pale green, with pale pinkish-purple stripes, and with conspicuous, black, elongate-oval chitinizations at the base of the body setae. Adults were reared September 30, 1923, and from February to April, 1924. The species overwinters as full-grown larvae.

NOCTUELIA RUFOFASCIALIS (STEPHENS)

Ennychia rufofascialis Stephens, 1834, Illus. Brit. Ent. Haust. 4: 33.

Noctuelia rufofascialis Barnes and McDunnough, 1918, Contrib. Nat. Hist. Lepidop. N. Amer. 4: 167; Heinrich, 1921, Jour. Agr. Research 20: 829-830.

Heinrich has recently received larvae of this species collected at Eagle Pass, Tex., by C. E. Bellis in cotton shipped from Rosita, Tex. This is a new host record. The insect has not been taken in the eastern part of Texas.

SUBFAMILY THYRIDINAE

MESKEA DYSPTERARIA GROTE

Meskea dyspteraria Grote, 1877, Canad. Ent. 9: 115; Dyar, 1902, List N. Amer. Lepidop. no. 4139; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 4897; Heinrich, 1921, Jour. Agr. Research 20: 828-829.

Galls in the stems of *Malvaviscus drummondii* Torrey and Gray containing larvae of this species were collected at Lake Charlotte September 21 and October 27, 1922, and October 23 and November 16, 1923. The galls were found mainly on small, stunted plants which made up one large colony. Other near-by colonies of the same plant, but of normal growth, were not at all infested. A few galls were found on *Malvaviscus* at Liberty, during September, 1923. They were usually formed singly on the upper half of the main stem, but occasional plants bore galls on the branches. In one case a very elongate enlargement on a branch of the host contained three larvae of *Meskea dyspteraria*, each in a separate cavity in the stem.

The conspicuous galls are of a light russet color, elongate-oval in shape, and usually quite regular in outline. They range in length from 20 to 50 mm., and in diameter at the middle or largest part of the gall from 6 to 13 mm., the average being about 40 mm. long and 11 mm. wide. In the upper part of the gall is a small round hole scarcely 1 mm. in diameter, through which frass in the form of short cylindrical pellets was discharged at regular intervals for a short period after the specimens were collected. Immediately after each operation the larva spun a web over the hole on the inside of the burrow. The burrow is lined with a thin web and is kept quite clean.

The winter is passed in the larval stage. When ready to pupate the larva cuts a round hole at the lower extremity of the cavity through the woody part of the gall to the outer surface, leaving the thin bark or epidermis unbroken. Pupation then takes place within the cavity, the caudal end of the pupa being fastened to the upper part of the burrow and the cephalic end pointing toward the newly constructed hole at the base of the cavity, through which the adult escapes upon emergence. The pupal case remains entirely concealed within the gall. Pupation took place during the latter part of March and the first part of April. From larvae collected September 21 and October 27, 1922, adults were reared April 17 to April 30, 1923, and from specimens collected October 23 and November 16, 1923, moths emerged April 13 to April 29, 1924.

Upon emergence the sluggish adults climbed to the upper part of the stems of the host plant in the rearing cages and rested in a very characteristic pose. In this pose the fore wings are more or less

folded along the anterior margin of the hind wings, which are spread, and in this position both pairs of wings are moved around toward the ventral surface of the insect until they form an acute angle with each other, but remain nearly at right angles to the thorax. Both sexes rest with the abdomen curved slightly upward.

Three specimens of the hymenopterous parasite *Calliephialtes grapholithae* (Cresson) were reared from larvae of *Meskea dyspteraria* Grote, each parasite developing singly within the body of its host and emerging through a small hole in one end of the gall. One specimen emerged on February 18 and two on March 12, 1924, from larvae collected at Lake Charlotte during November, 1923.

SUBFAMILY PHYCITINAE

HOMOEOSOMA ELECTELLUM (HULST)

Anerastia electella Hulst, 1887, Entomologica Americana 3: 137-138.
Homoeosoma electellum Hulst in Dyar, 1902, List N. Amer. Lepidop., no. 4865; Heinrich, 1921, Jour. Agr. Research 20: 832.

Two adults were reared September 11 and October 5, 1922, from larvae found feeding in the flower heads of *Helianthus* sp. at Smith Point August 9 and September 14. From larvae collected in the flower heads of *Rudbeckia maxima* Nuttall and *Helianthus* sp. at Liberty in June and July, 1923, adults were reared June 27 to July 16 of the same year. Pupation took place in the irregular webbed channels made by the larvae.

From a parasitized larva taken in a flower head of *Rudbeckia maxima* a specimen of *Spilochalcis delira* (Cresson) was reared July 5, 1923.

EUZOPHERA SEMIFUNERALIS (WALKER)

Nephteryx semifuneralis Walker, 1863, List Lepidop. Brit. Mus. 27: 57.
Euzophera semifuneralis Hulst, 1890, Trans. Amer. Ent. Soc., 17: 175; Dyar, 1902, List N. Amer. Lepidop., no. 4832; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5720.

Four adults were reared March 18 to April 14, 1924, from larvae collected in stalks of corn at Snyder, Scurry County, December 19, 1923. The larvae construct rather heavy webs in the irregular burrows in the pith of the host for pupation.

EPHESTIA CAUTELLA (WALKER)

Pempelia cautella Walker, 1863, List Lepidop. Brit. Mus. 27: 73.
Ephestia cautella Rebel, 1901, Cat. Lep. Eur. 2, no 271; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5801.

Adults of this species were reared from larvae found feeding upon the pulp of ripened bolls of *Malvaviscus drummondii* Torrey and Gray collected at Lake Charlotte during August, 1922. The larvae pupated within folds in small pieces of cloth in the rearing cages. Emergence dates ranged from October 7 to October 17, 1922, the pupation period averaging 10 days.

FAMILY COSMOPTERYGIDAE

CHAETOCAMPA BUSCK,¹ NEW GENUS

(Fig. 1, A to F; fig. 2, D; fig. 3, A, B, C)

Type.—*Chaetocampa crotonella* Bottimer, hereinafter described.

Chaetocampa, n. g.

Labial palpi curved upward, sickle shaped, smooth, reaching vertex, terminal joint nearly as long as second and pointed. Antennae with strong pecten on basal joint. Face and head smooth. Fore wings narrow lanceolate; 12 veins; 6 and 7 long-stalked, inclosing apex; 8 out of their stalk; 5 out of base of stalk; rest separate; 1b not furcate at base; 1c present, strong. Hind wings half as wide as fore wings; lanceolate; 8 veins; 6 and 7 very long-stalked or united; rest separate; 5 nearest 6 and 7. A series of long spinelike scales along costal edge. Posterior tibiae strongly tufted above. Male genitalia with rounded broad uncus; gnathos divided into two free knobbed arms; socii absent; tegumen slender, elongate; harpes greatly reduced, small, semicircular; vinculum narrow, with long anteriorly projecting process; annellus strongly developed, with chitinous processes; aedoeagus long, slender, and nearly straight. Larva with secondary setae.

The genus is for the present placed in the family Cosmopterygidae, where it would go on its oral and pterogostic characters, but the larvae and the genitalia prove that this family is heterogeneous and must be divided. The present genus does not go with *Cosmopteryx* and its allies, but represents a distinct family, characterized by the quite different genitalia and by the hairy larvae. (Fig. 3, C.)

Belonging to this family and closely allied to the present genus is the Transcaucasian genus *Parametriotes* Kusnezov (type, *P. theae* Kusnezov),⁵ and some of the species at present wrongly referred to the genus *Batrachedra* Stainton.

The definition of the family is advisedly postponed until the entire group can be properly worked up.

CHAETOCAMPA CROTONELLA, NEW SPECIES

Chaetocampa crotonella, n. sp.

Antenna above whitish ochreous; beneath black, pubescent, and with whitish ochreous scales at apex of each joint, giving under surface a serrate appearance. Labial palpus whitish ochreous; second joint with a few dark fuscous scales on upper and outer sides, especially near apex; terminal joint with a few dark fuscous scales in middle on outer side. Face, head, and thorax whitish ochreous. Fore wing whitish ochreous, overspread with dark fuscous scales, more strongly above and beyond cell; a distinct black dot at end of cell; from there an indistinct longitudinal dark streak to apex, and a more or less distinct marginal series of black dots; cilia light ochreous fuscous. Hind wing dark silvery fuscous; cilia light ochreous. Under surface of both fore and hind wings dark silvery fuscous. Abdomen whitish ochreous. Legs whitish ochreous, shaded with light fuscous exteriorly.

Male genitalia of paratype from Smith Point figured (fig. 1, D, E).

Alar expanse.—9 to 13½ mm.

Type.—Cat. No. 27333, United States National Museum.

Type locality.—Liberty, Tex.

Food plant.—*Croton engelmannii* Ferguson.

Described from male type (May 27, 1924) and three male and four female paratypes reared April 9 to June 16, 1924, from larvae collected in seeds October 19, 1923; one male and two female paratypes

¹ Description of the new genus furnished by August Busck.

⁵ KUSNEZOV, N. J. DESCRIPTION OF PARAMETRIOTES THEAE, GEN. N., SP. N. (LEPIDOPTERA, TINEIDAE), A NEW ENEMY OF THE TEA BUSH IN TRANSCAUCASIA. Russ. Ent. Obozr. (Rev. Russe Ent.) 15: 627-652, illus. 1916. [In Russian. English summary, p. 642-649.]

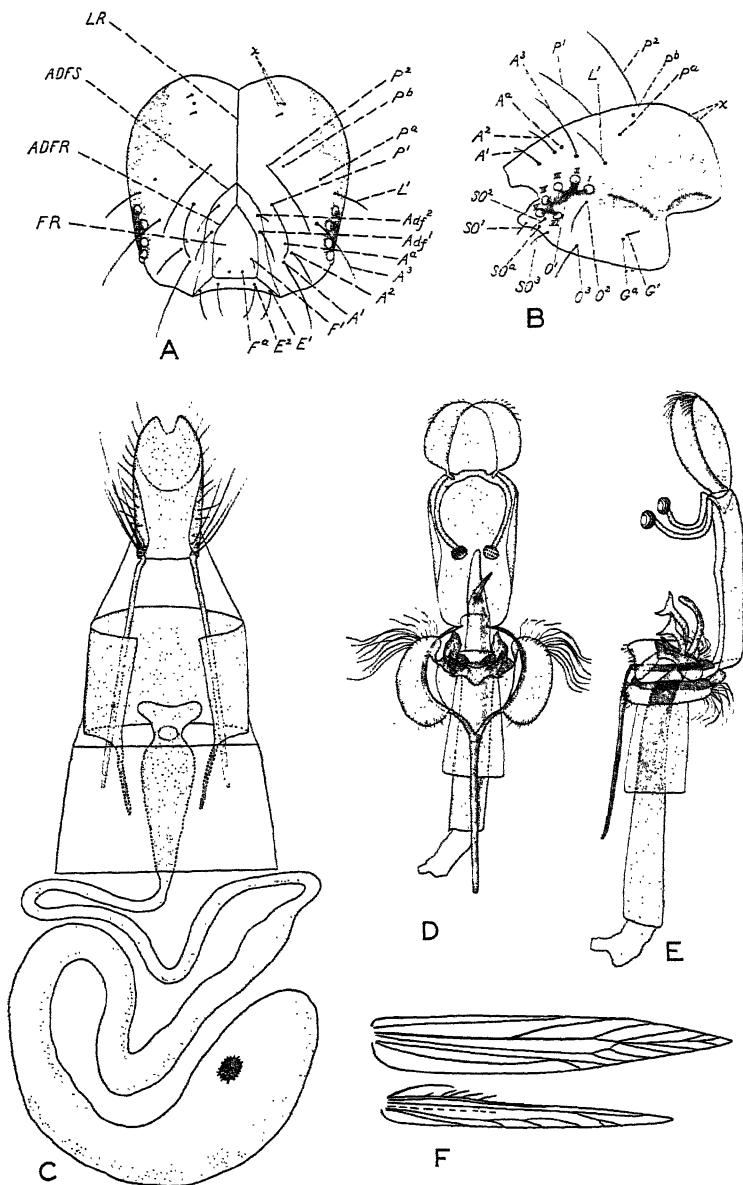


FIG. 1.—*Chaetocampa crotonella*: A, head of larva, front view; B, head of larva, side view; C, female genitalia; D, male genitalia, front view; E, male genitalia, side view; F, wing venation

A¹, A², A³, A*, anterior setae and puncture of epicranium; Adf¹, Adf, adfrontal setae and puncture of epicranium; ADfR, adfrontal ridge of frons; ADfS, adfrontal suture; E¹, E², epistomal setae; F¹, F*, frontal seta and puncture; FR, frons; G¹, G*, genal seta and puncture of epicranium; L¹, lateral seta and puncture of epicranium; LR, longitudinal ridge of frons; O¹, O², O³, ocellar setae and puncture of epicranium; P¹, P², P³, P⁴, posterior setae and punctures of epicranium; SO¹, SO², SO³, SO*, subocellar setae and puncture of epicranium; X, ultraposterior setae and punctures of epicranium.

reared July 28, 1923, from larvae collected in leaf galls July 6, 1923, all from the type locality; and one male paratype reared August 31, 1922, from a leaf gall found at Smith Point, Tex.

Chaetocampa crotonella is quite common in parts of Liberty and Chambers Counties. Larvae were collected in galls in the petioles of leaves of the host plant from June 28 to August 10. The galls are usually formed next to the leaf blade and are somewhat irregularly conical in shape, tapering away from the base of the leaf blade. The enlargements average from 3 to 4 mm. in diameter at the widest point and range from 5 to 15 mm. in length.

Later in the season the larvae are to be found only in the immature three-seeded capsules of the same plant. Larvae were noted in the seeds as early as July 11, and were collected as late as November 13 in late-maturing plants. In the capsule the larva feeds upon the contents of the seeds, going from one to another through very clean-cut holes.

The larva possesses secondary body setae. The younger stages are white. When full grown the larva is 8 to 10 mm. long and purple-black, the color becoming paler toward the anterior end; thoracic segments each with a middle lateral spot of the darker color. Head pale purplish white, with lateral margins purple-black; thoracic shield purplish white with darker spots, posterior margin broadly purple-black; thoracic legs pale; abdominal legs normal, crochets (fig. 2, D) about 15 in number, arranged in a circle broken outwardly; anal plate of body color; anal fork absent.

Pupation of the larva found in the galls takes place within the enlarged petioles of the leaf, the pupal case remaining in the gall after the emergence of the moth. When full grown the seed-feeding larva leaves the capsule of the host and seeks a suitable place in which to pupate, but does not enter the soil. Those in the rearing cages pupated in pieces of cork, the cases remaining in the silk-lined cavities upon emergence of the adults. The species overwinters as pupae.

The pupa (fig. 3, A, B) is 5 to $6\frac{1}{2}$ mm. long and 1 to $1\frac{1}{4}$ mm. wide; nearly cylindrical, slightly dorsally concave; color yellowish brown; surface granular, dorsal surface of abdominal segments finely transversely corrugated. Front, vertex, prothorax, and anterior half of mesothorax thickly covered with very short, stout spines, and with a few larger scattered spines; a large curved spine at lower margin of eye, several on first joints of antennae, one on outer hind angle of vertex, several on posterior half of mesothorax, and one near outer margin of metathorax. Maxillae and prothoracic legs about half as long as wing cases; antennae, metathoracic legs, and wing cases reaching beyond fifth abdominal segment; serrations of antennae plainly visible; antennae meeting at fourth abdominal segment and diverging posteriorly, exposing the tips of metathoracic legs. Abdomen with movable joints between segments 5 and 6, and 6 and 7, the sutures between these segments being wide and deep; caudal end bluntly pointed; segments 9 and 10 fused ventrally; anal prolegs with crochets present.

Larvae found in the galls produced moths July 13 to August 31 of the same year. A few adults were reared during the later part of September from larvae taken in seeds, but most of the seed-feeders did not produce moths until the following year, the emergence dates

ranging from April 5 to June 17. The adult rests with all its legs touching the surface, with the head slightly elevated, and with the antennae above the wings along the side of the body.

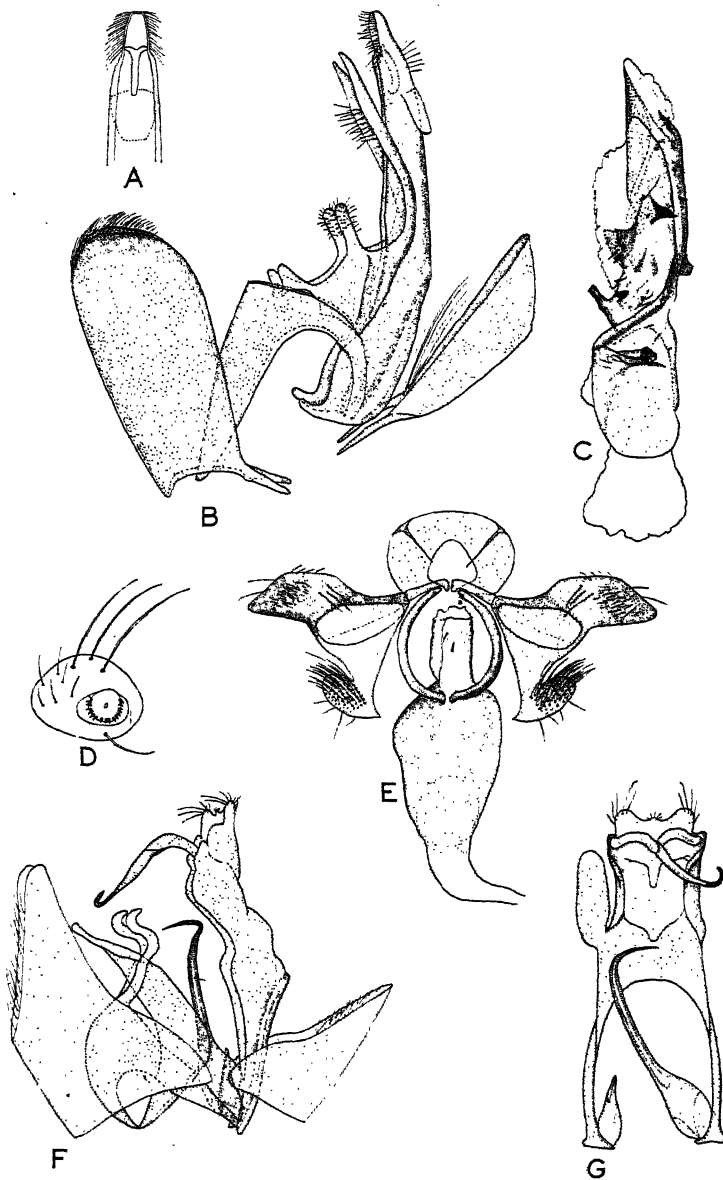


FIG. 2.—*Gelechia monotaeniella*: A, male genitalia, uncus; B, male genitalia, aedeagus removed; C, male genitalia, aedeagus. *Chaetocampa crotonella*: D, abdominal proleg of larva. *Isophrictis similtiella*: E, male genitalia. *Recurvearia eryngiella*: F, male genitalia, side view; G, male genitalia, front view.

Two specimens of *Bassus gibbosus* Say (Hymenoptera) were reared January 14, 1924, from Croton seed capsules collected at Liberty and are probably parasitic upon the lepidopteron.

FAMILY GELECHIIDAE

ISOPHRICTIS SIMILIELLA (CHAMBERS)

(Fig. 2, E; fig. 3, D, E, F)

Gelechia similiella Chambers, 1872, Canad. Ent. 4: 193.*Paltodora similiella* Busck, 1903, U. S. Natl. Mus. Proc. 25: 779-780.*Isophrictis similiella* Meyrick, 1917, Ent. Mo. Mag. 53: 113; Heinrich (in part), 1921, Jour. Agr. Research 20: 813-814.

In Heinrich's paper two species of *Isophrictis* are confused under the name *similiella*—the true *similiella* (Chambers) and *rudbeckiella*, a species here described as new, the genitalia figured in Plate 95, A, of his paper belonging to the new species.⁶ Both species have the harpe of the male genitalia divided, but in the new species it is nearly twice as long as in *similiella*.

Five adults were reared from September 26 to October 21, 1922, from larvae collected in the flower heads of a tall wild sunflower (*Helianthus* sp.) at Smith Point in September. The larvae were found in large numbers in the dried flower heads of a similar sunflower at Sweetwater and San Angelo, in northern Texas, during December. Specimens collected at the latter locality December 14, 1923, remained over winter as larvae. In the rearing cage pupation took place from May to June, the adults emerging from May 7 to July 1, 1924.

The larvae range from 5 to 10 mm. in length and have three or four crochets on the anal and abdominal legs. The pupa (fig. 3, D, E, F) is 5 to 6½ mm. long, 1¼ to 1½ mm. wide; cephalic end pubescent; wing cases reaching nearly to eighth abdominal segment; tips of metathoracic legs exposed beyond tips of wing cases; maxillae extending well beyond caudal end, which is armed with numerous elongate hooked spines and a dorsally projecting cremaster.

From the San Angelo material the following Hymenoptera were reared during April, 1924: *Microbracon mellitor* (Say), one specimen; *M. nuperus* (Cresson), one specimen; *Microbracon* n. sp., one specimen; *Zaglyptonotus schwarzi* Crawford, three specimens; *Callimome* sp., three specimens; and four specimens of a pteroma id. Six adults of *Cremastus facilis* (Cresson) were also obtained, the emergence dates ranging from April 27 to July 14, 1924.

ISOPHRICTIS SIMILIELLA DENOTATA, NEW VARIETY

(Fig. 2, G, H)

Isophrictis similiella denotata, n. v.

Differs from the true *similiella* in its darker color, and in having the fore wings covered with white-tipped black scales without the distinct golden longitudinal streaks. These are replaced by scattered reddish brown scales, which at the apex form a v-shaped mark as in the typical specimens.

The genitalia are the same as in *similiella*.

Alar expanse.—9 to 16 mm.

Type.—Cat. No. 27334, United States National Museum.

Type locality.—Liberty, Tex.

Food plant.—*Helianthus mollis* Lamareck.

Described from male type and 4 male and 11 female paratypes reared September 17 to October 24, 1923, from larvae collected during the first part of the same month.

⁶ HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 807-836, pl. 95, A. 1921.

The habits of the larva are similar to those of the larva *Isophrictis similiella*. The larva differs from that of the true *similiella* in having one crochet, rarely two, on the anal and abdominal legs. The pupa

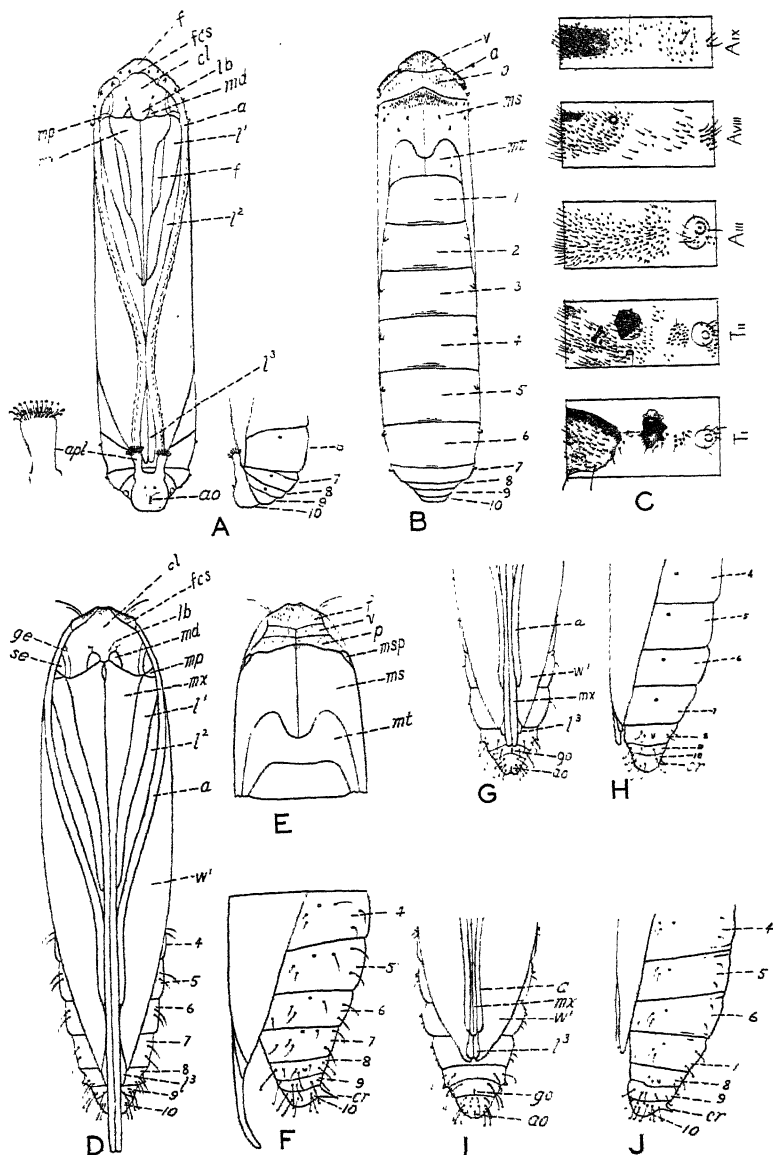


FIG. 3.—*Chaetocampa crotonella*: A, pupa, front and side views; B, pupa, back view; C, setal map of larva. *Isophrictis similiella*: D, pupa, front view; E, pupa, back view; F, pupa, side view. *Isophrictis similiella denotata*: G, posterior tip of pupa, front view; H, posterior tip of pupa, side view. *Isophrictis rudbeckiella*: I, posterior tip of pupa, front view; J, posterior tip of pupa, side view.
a, antenna; ao, anal opening; apl, anal proleg; cl, clypeus; cr, cremaster; f, front; f¹, femora of prothoracic leg; fcs, fronto-clypeal suture; ge, glazed eye; go, genital opening; l¹, prothoracic leg; l², mesothoracic leg; l³, metathoracic leg; lb, labrum; md, mandible; mp, maxillary palpus; ms, mesonotum; msp, mesothoracic spiracle; mt, metanotum; mx, maxilla; p, pronotum; se, sculptured eyepiece; v, vertex; w¹, mesothoracic wing

(fig. 3, G, H) differs in the length of the maxillae, which extend only slightly beyond the tips of the wing cases and metathoracic legs; in none do they reach the tip of the abdomen.

Twelve specimens of *Tachinophyto* sp. (Diptera) were reared September 18 to October 24, 1923, as parasites of the larvae of this species.

During the latter half of September, 1923, the following Hymenoptera were reared from the Liberty material: *Apanteles dakotae* Muesebeck, four specimens; *Callimome* sp., one specimen; and *Cremastus* sp., one specimen.

ISOPHRICTIS RUDBECKIELLA, NEW SPECIES

(Fig. 3, I, J)

Isophrictis rudbeckiella, n. sp.

Antenna with basal joints whitish, overlaid with fuscous scaling; apically becoming golden ochreous, strongly annulated with black, the ochreous scales spreading, giving a serrated appearance, especially on apical half; finely pubescent beneath. Palpus white; second joint with outer upper edge fuscous, and with outer side and inner apical third of well-developed brush fuscous; terminal joint with outer side fuscous, extreme tip black. Face white. Head and thorax covered with white-tipped dark scales. Fore wing covered with white-tipped dark scales, the darker color fuscous at base, becoming black at apex of wing; marked with narrow longitudinal streaks of white-tipped golden scales, one below costa, one along fold, and a third midway between the two, rather distinct on basal half, the streaks beyond middle becoming shorter, more numerous, and confused, forming a rather distinct V-shaped area of golden scales pointing toward the apex just before a strong edging of white-tipped black scales; cilia light fuscous, at apex darker, and crossed by two nearly black lines. Hind wing dark silvery fuscous; cilia light fuscous. Both fore and hind wings dark fuscous beneath. Abdomen dark silvery fuscous, with silvery lateral streak. Legs silvery white, heavily overlaid with fuscous exteriorly.

Alar expanse.— $11\frac{1}{2}$ to 16 mm.

Type.—Cat. No. 27335, United States National Museum.

Type locality.—Liberty, Tex.

Food plant.—*Rudbeckia maxima* Nuttall. Also reared from *R. hirta* Linnaeus.

Described from male type and four male and six female paratypes reared June 10 to June 28, 1924, from larvae collected in flower heads of *Rudbeckia maxima* at Liberty September, 1923, and one male paratype reared from *Rudbeckia* sp. (Heinrich, May 26, 1918) at Alvin, Brazoria County.

This species is distinguished from *similiella* Chambers only by means of the male genitalia, slight but constant larval and pupal characters, and host plants. The male genitalia of *rudbeckiella* are figured by Heinrich⁷ under the name *Isophrictis similiella* Chambers.

The larva feeds throughout the flower head of its host and pupates within the burrow or in the upper part of the stem. From that of *similiella* the larva differs in having one or two crochets on the anal legs and none on the abdominal legs. The pupa (fig. 3, I, J) differs in having the maxillae considerably shorter than the wing cases, which are equal in length to the metathoracic legs and extend slightly beyond the seventh abdominal segment.

Two generations were noted. Larvae and pupae collected in *Rudbeckia maxima* on July 17, 1922, produced adults from July 26 to August 12, 1922, and larvae taken in the same host in September,

⁷ HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 807-836, pl. 95, A. 1921.

1923, produced moths the following June. A large series of the second generation was reared during June, 1924, from flower heads of *Rudbeckia hirta* L. naeus taken at Liberty in November of the previous year. The winter is passed in the larval stage.

The larvae were found to be parasitized by several Hymenoptera. From those in *Rudbeckia maxima* three specimens of *Aerophilus* n. sp. were reared April 3, April 8, and June 11, 1924, and one specimen of *Cremastus* sp. emerged March 26 of the same year. Sixteen specimens of *Aerophilus* n. sp. (March 31 to June 19), one specimen of *Microbracon* n. sp. (May 13), and two specimens of *Cremastus minor* Cushman (April 30) were obtained during 1924 from the larvae in *R. hirta*.

The species recorded by Braun⁵ as *Isophrictis similiella* Chambers is presumably referable to the present species.

ARISTOTELIA CORALLINA WALSINGHAM

Aristotelia corallina Walsingham, 1909, Biol. Centr.-Amer., Lep.-Het. 4: 23.

Larvae of this species were collected on leaves of *Vachellia farnesiana* (Linnaeus) Wight and Arnot at Lake Charlotte, in September and October, 1923. From this material a series of 12 adults emerged October 4 to October 13, 1923, and January 11 to January 12 of the following year. This is the first record of this Mexican species occurring in the United States.

The larvae construct silken tubes, to which are fastened leaflets of the host plant. The full-grown larva is 8 to 10 mm. long. Body with second and third thoracic segments broadly banded dorsally, with purplish black, pale lavender beneath, intersegmental areas white; first abdominal segment with anterior half white, posterior half purplish black; second to fourth abdominal segments with posterior dorsal third purplish black, anterior dorsal third same color but marked with broad wavy white bands, middle dorsal third pale lavender with similar white markings; bands of first to fourth abdominal segments becoming paler beneath; fifth to tenth abdominal segments beneath pale brownish, more or less mottled with white, dorsally purplish black except for narrow dorsal whitish stripe from anterior margin of sixth abdominal segment to caudal end; a similar broad whitish lateral stripe extending from just before middle of fifth abdominal segment to caudal end; the three whitish bands mottled with pale lavender, becoming pale brown on last segment. Thoracic legs black; abdominal legs slightly elongate, crochets numerous, arranged in a complete circle; anal plate with lateral margins broadly brownish black, middle stripe whitish; anal fork well developed, two-pronged, black.

The pupa is 4 to 4½ mm. long, about 1 mm. wide; dark reddish brown; entirely pubescent; antennae, metathoracic legs, and wing cases reaching nearly to posterior margin of fifth abdominal segment; caudal end with numerous long, hooked spines, and cremaster.

⁵ BRAUN, A. F. NOTES ON MICROLEPIDOPTERA WITH DESCRIPTIONS OF NEW SPECIES. Ent. News 32:8. 1921.

LATHONTOGENUS PALPIGERA WALSINGHAM

- Gelechia palpigera* Walsingham, 1891, Ent. Soc. Lond. Trans., p. 94, pl. 4, fig. 31.
Lathontogenus adustipennis Walsingham, 1897, Zool. Soc. Lond. Proc. 1: 88.
Paraspistes ioloncha Meyrick, 1905, Jour. Bombay Nat. Hist. Soc. 16: 600; 1911, Linn. Soc. Lond., Trans. (2), 14: 274.
Lipatia crotalariella Busek, 1910, Trinidad Dept. Agr. Bul. 9: 243, 244.
Paraspistes palpigera Busek, 1914, U. S. Natl. Mus. Proc. 47: 10, 11.
Lathontogenus palpigera Walsingham, 1915, Biol. Centr.-Amer., Lep.-Het. 4: 409.

A single adult was reared March 9, 1923, from pods of *Vachellia farnesiana* (Linnaeus) Wight and Arnot collected at Kingsville during January, 1923. The species is known from the Tropics, and has not heretofore been recorded from the United States.

RECURVARIA ERYNGIELLA, NEW SPECIES

(Fig. 2, F, G)

***Recurvaria eryngiella*, n. sp.**

Antenna golden ochreous, basal joint white with small black spot above. Labial palpus white with dark fuscous scales on outer side at base; terminal joint with two broad black annulations, one near base and the other near tip. Face and head white. Thorax white with base of patagium and posterior dot black. Forewing white, strongly overlaid on outer two-thirds with golden ochreous scales, especially below the black costal markings; costal edge with small black streak at base, narrowly black to the middle, from which a short, outwardly oblique, black streak runs toward a few black scales on end of cell; at apical third a similar smaller, outwardly oblique, black streak; a few scattered black scales at apex; a black spot below middle of fold, and a smaller black dot near end of dorsum; cilia golden white, with scattered black dots near apex. Hind wing light silvery fuscous; cilia white. Abdomen and a strong hair pencil at base of hind wing golden ochreous. Legs golden ochreous, with broad black bars and annulations, the darker markings less pronounced on the hind legs.

Male genitalia of type figured (fig. 2, F, G).

Alar expanse.—7 to 10 mm.

Type.—Cat. No. 27336, United States National Museum.

Type locality.—Stowell, Chambers County, Tex.

Food plant.—*Eryngium aquaticum* Linnaeus.

Described from male type and 17 male and 34 female paratypes reared July 11 to July 16, 1923, from larvae and pupae collected July 3, 1923.

This species is near *variella* Chambers and *apicitripunctella* Clemens. From the former it differs in having the labial palpi and the legs marked with black. From the latter it is distinct in having the face, head, and thorax white, and in lacking the annulations on the antennae.

As many as 10 larvae were removed from a single flower head in which feeding had taken place. Pupation occurs within the silk-lined channels made by the larvae, the pupal cases remaining in the cavities after emergence of the adults. The full-grown larva is 7 to 8 mm. long; body color white, each segment with a broad, deep pink band on posterior half of dorsal surface, the white of the anterior half of thoracic segments 2 and 3 and abdominal segments 1 to 4 quite distinct, but that of the other abdominal segments becoming narrower and less distinct posteriorly; under surface similarly banded but paler. Head pale yellowish brown; thoracic shield slightly lighter in color, usually with a darker lateral border on each side; chitinizations at base of body setae small, round, yellowish in color; thoracic legs yellowish; crochets of abdominal legs numerous, arranged in a circle broken inwardly; anal plate yellowish brown; anal fork well developed, eight-pronged.

The pupa is about 4 mm. in length and 1 mm. in width; cephalic end bluntly rounded, abdomen gradually tapering posteriorly, caudal end rather pointed, armed with numerous elongate hooked spines, but without cremaster; maxillae more than half the length of wing cases; wing cases, antennae, and metathoracic legs nearly reaching sixth abdominal segment; antennae with tips separated, exposing ends of metathoracic legs.

RECURVARIA ROBINIELLA (FITCH)

Anacampsis robinielli Fitch, 1859, Rept. Nox. Benef. Ins. N. Y. 5: 834-835.
Recurvaria robinielli Busck, 1903, U. S. Natl. Mus. Proc. 25: 812, 813.

Twelve adults were reared March 17 to April 4, 1924, from larvae collected on *Amorpha fruticosa* Linnaeus at Lake Charlotte October 23, 1923. Feeding and pupation of the larva took place between two leaflets, which were webbed together along the edge so that the upper surfaces were on the inside. The species overwinters as pupae.

POLYHYMNO ACACIELLA BUSCK

Polyhymno acaciella Busck, 1900, Jour. N. Y. Ent. Soc. 8: 235-236, pl. 9, fig. 1; Busck, 1903, U. S. Natl. Mus. Proc. 25: 839; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 6179.

One adult was reared October 2, 1923, from leaves of *Vachellia farnesiana* Wight and Arnot collected in September, 1923, at Lake Charlotte.

GELECHIA MONOTAENIELLA, NEW SPECIES

(Fig. 2, A, B, C)

Gelechia monotaeniella, n. sp.

Antenna purplish black, with indistinct white annulations. Labial palpus with second joint whitish, dusted with black scales exteriorly; brush rather short, furrowed, gradually diminishing toward apex of second joint; terminal joint black, with few scattered white scales. Face, head, and thorax heavily overlaid with purplish-black scales. Fore wing dark fuscous, with few scattered black and brown scales; at apical fourth a transverse, irregular, incomplete, whitish fascia, before which are an ill-defined black costal spot and a similar dorsal spot; cilia light fuscous, with edging of black scales at apex. Hind wing silvery fuscous, dark toward apex; cilia light fuscous. Abdomen with basal segments above velvety yellowish, others above fuscous; under surface very dark fuscous. Legs dark fuscous, with tips of tarsi white.

Male genitalia of types figured (fig. 2, A, B, C).

Alar expanse.—12 to 16 mm.

Type.—Cat. No. 27337, United States National Museum.

Type locality.—North shore of Lake Charlotte, Chambers County, Tex.

Food plant.—*Vachellia farnesiana* (Linnaeus) Wight and Arnot.

Described from male type (Nov. 12, 1923) and 2 male and 16 female paratypes reared October 13 to November 12, 1923, and January 29 to March 24, 1924, from larvae collected from September to November, 1923.

This species is very near *serotinella* Busck, from which it is distinguished by the conspicuous whitish fascia on the fore wing, by lacking the narrow, chocolate brown, longitudinal streak below the costal edge of the fore wing (which is prominent in *serotinella*), and by its smaller size.

The larva is a leaf feeder, constructing a white silken tube along the midrib of a leaf or along a small branch and fastening to it leaflets of the host. The full-grown larva is 13 to 15 mm. long. Body pale

greenish white; upper surface with six broad, very irregular, wine-colored stripes, two dorsal, a subdorsal, and a lateral, the subdorsals being slightly widest; ventral surface with three similar but much paler stripes, a narrow ventral and a broad, poorly defined marking on each side just above prolegs. Head pale yellow, posterior half reddish brown, with irregular, elongate, triangular markings of the same color projecting anteriorly; posterior margin narrowly black. Thoracic shield pale yellow, usually indistinctly bordered with black on posterior margin. Thoracic legs and anal plate yellow; abdominal legs normal, with alternating long and short crochets arranged in a complete circle; anal fork absent.

Pupation took place within thin, white, oval cocoons, to the outside of which were fastened particles of frass and leaflets of the host. A few of the specimens passed the winter as pupae, but most of them did not pupate until the first part of February. The colored stripes of the wintering larvae are barely visible.

The pupa is smooth, rather stout, 5 to 6 mm. long, and $1\frac{3}{4}$ to 2 mm. wide at the metathorax or widest part; cephalic end bluntly rounded; abdomen gradually tapering to caudal end, which is also bluntly rounded; maxillae very broad at base, two-thirds as long as wing cases; antennae meeting beyond maxillae but abruptly converging before end of wing cases, exposing ends of metathoracic legs; antennae, metathoracic legs, and wing cases reaching nearly to sixth abdominal segment; ventral posterior margin of seventh abdominal segment scalloped, and fringed with very short hairs; caudal end without cremaster.

A dipteran, *Zenillia blanda* Osten Sacken, emerged March 22, 1924, from a pupa of *Gelechia monotaeniella*. The parasite had pupated within the pupa of its host. A second parasitized pupa was noted, but the dipteran was not reared.

Two specimens of the hymenopteron *Brachymeria hammari* (Crawford) emerged October 8 and October 9, 1923, from two pupae of *Gelechia monotaeniella*. They were undoubtedly secondary parasites of the gelechiid, the primary host in this case being *Zenillia blanda*.

FAMILY BLASTOBASIDAE

ZENODOCHIUM CITRICOLELLA (CHAMBERS)

Blastobasis citricolella Chambers, 1880, U. S. Com. Agr. Rept. 1879: 206-207.
Zenodochium citricolella Dietz, 1910, Amer. Ent. Soc. Trans. 36: 11-12.

Several larvae and pupae of this species were taken in mature capsules of jimson weed (*Datura tatula* Linnaeus) at Smith Point, August 11, 1922. The larvae were webbing together and feeding upon the seeds. The pupae were in thin but strong webs, to the outside of which were fastened particles of frass and seeds. From this material five adults were obtained, the emergence dates ranging from August 17 to September 11, 1922.

HOLCOCERA CONFAMULELLA HEINRICH

Holcocera confamulella Heinrich, 1921, Jour. Agr. Research 20: 818-819.

Eight adults were reared during April, 1923, from fruits of *Crataegus* sp. collected at Smith Point October 18 and November 1, 1922.

FAMILY OLETHREUTIDAE

SUBFAMILY LASPEYRESIINAE

LASPEYRESIA PACKARDI (ZELLER)

- Grapholitha packardi* Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 300.
Steganoptycha pyricolana Murtfeldt, 1891, U. S. Dept. Agr. Bul. 23: 52.
Epinotia pyricolana Fernald, in Dyar, 1902, List N. Amer. Lepidop., no. 5234.
Enarmonia packardi Fernald, in Dyar, 1902, List N. Amer. Lepidop., no. 5282.
Enarmonia pyricolana Garman, 1918, Md. Agr. Expt. Sta. Bul. 223: 105-106, 108-109.
Laspeyresia packardi Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 7238.

A single male specimen was obtained April, 1923, from fruits of *Crataegus* collected at Smith Point during October of the preceding year. The above synonymy was furnished by Carl Heinrich.

SUBFAMILY EUCOSMINAE

EUCOSMA GIGANTEANA MINORATA HEINRICH

Eucosma giganteana minorata Heinrich, 1924, Jour. Wash. Acad. Sci. 14: 388.

In each of two flower heads of *Silphium gracile* A. Gray was found a dirty pinkish larva which might easily be mistaken for the pink bollworm. The part of the flower head above the cavity eaten out by the larva had darkened in color, indicating the presence of the insect. The specimens were collected near Liberty, July 17, 1922. Pupation of the one specimen reared took place July 19 in the cavity made during the growth of the larva and within a thin cocoon covered with small pieces of the dead portion of the flower head. The adult emerged July 29, 1922.

A second adult emerged July 5, 1923, from a pupa taken in a typically injured flower head of *Silphium gracile* at Stowell, Chambers County, July 3 of the same year. From material collected in mature and immature flower heads of the same plant at Liberty, July 7, 1923, a third moth was reared nine days later.

These adults furnished the type material for the variety *minorata* Heinrich.

EPIBLEMA TRIPARTITANA (ZELLER)

- Paedisca tripartitana* Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 308.
Eucosma tripartitana Fernald, in Dyar, 1902, List N. Amer. Lepidop., no. 5141; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 7025.
Epiblema tripartitana Heinrich, 1923, U. S. Natl. Mus. Bul. 123: 146.

This is the olethreutid referred to in Heinrich's paper.⁹ The host plant of this species is *Rudbeckia maxima* Nuttall, commonly called "niggerhead" in eastern Texas. The larvae are quite common in parts of Liberty, Chambers, and Harris Counties. The larva spends the first part of its life in a burrow in the central part of the flower head of the host plant, the cavity extending downward about an inch into the stem. The presence of the larva may often be detected by a slight enlargement of the stem immediately below the flower head.

⁹ HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 813, illus. 1921.

The taking of a single specimen at light (Liberty, July 23, 1924) and the presence of a very few pupal cases in the flower heads of the hosts during July indicate a possibility of there being two generations. If such is the case, the following notes pertain to the second generation.

When apparently full grown the larva leaves its host through a small hole near the bottom of the cavity in the upper part of the stem and enters the same plant about a half inch above one of the lower leaves, from 6 to 12 inches above the surface of the soil. The leaf is webbed to the stem at the point of entrance of the larva. The conspicuous white chewings which have fallen to the ground at the base of the plant indicate that the larva has recently entered the stem. Upon entering the lower part of the plant the larva constructs a burrow which extends from a point 2 or 3 inches above the entrance hole to the base, where the cavity is slightly enlarged. The entrance hole is then plugged securely.

A few of the larvae had made the change described above before June 23, and by July 15 about half of them had reentered the plant at the base. Larvae were found in the flower heads of the host as late as October 10.

This insect passes the winter in the larval stage at the base of the host plant. At some time during the winter the larva cuts the stem off from the inside at the upper part of its cavity and plugs the upper inch or so with chewings. Just before pupation a hole is chewed nearly through the stem in the uppermost part of the remaining cavity for the emergence of the moth. Pupation takes place in the burrow. A number of pupae were observed to move quite rapidly down the burrow by means of a spiral movement of the abdomen.

The first pupa was taken at Liberty February 23, 1924, and from it the adult emerged March 15, 1924. From plants collected at the above locality on February 23 and 27, 1924, adults emerged March 15 to April 29 of the same year.

The larvae were parasitized by two species of Diptera: *Siphophyto floridensis* Townsend, four specimens of which were reared June 28 to July 3, 1923, and *Tachinophyto* sp., two specimens of which were reared June 29 and July 3, 1923.

The larvae were also parasitized by *Macrocentrus* sp. (Hymenoptera), the brownish cocoons of which were taken in large numbers in the flower heads of the host plant during June and July. Adults were reared June 29 to July 10. From a parasitized larva in the lower part of the stem of the host one specimen of *Bassus* sp. was reared March 29, 1924. The hymenopteron had spun its cocoon within the burrow made by the host.

EPIBLEMA DISCRETIVANA (HEINRICH)

Eucosma discretivana Heinrich, 1921, Jour. Agr. Research 20: 823-824.

Epiblema discretivana Heinrich, 1923, U. S. Natl. Mus. Bul. 123: 138, 147-148.

Larvae of this species were taken in stem galls of "wild myrtle" in August, 1924, with the assistance of A. C. Johnson, who collected and reared the type material of the species. The larvae were collected at Sheldon, Harris County, the type locality. The host plant has been determined as *Baccharis halimifolia* Linnaeus, of the family Carduaceae.

CROCIDOSEMA PLEBEIANA ZELLER

Crocidosema plebeiana Zeller, 1847, Isis von Oken 10: 721-722; Heinrich, 1921, Jour. Agr. Research 20: 822-823.

Larvae of this species were collected in several Malvaceae in addition to those mentioned by Heinrich.¹⁰ From capsules of *Sida* sp. collected at Liberty July 31, 1922, several adults emerged during the first part of the following month. At Lake Charlotte on November 6 larvae were found in large numbers in the seed pods of *Sida spinosa* Linnaeus, *S. rhombifolia* Linnaeus, *Malvastrum americanum* (Linnaeus) Torrey, and *Anoda cristata* (Linnaeus) Schecht. Adults were reared during November, 1923, from larvae collected in mature capsules of *Reidlea corchorifolia* (Linnaeus) De Candolle at Smith Point November 1, 1923. This host plant belongs to the Buettneriaceae, and closely resembles some of the malvaceous plants.

The larvae were found singly in the seed capsules, except in the case of okra, in which several specimens were taken in one pod. In *Sida* and other host plants having small seed pods the larvae were often taken in a tie of the terminal leaves and immature capsules. The larvae pupated where feeding had taken place or within a roll or fold at the edge of a leaf. There are evidently two or more generations a year. Emergence dates ranged from the middle of August to the first part of December.

EPINOTIA PERPLEXANA (FERNALD)

Epiblema perplexana Fernald, 1901, Jour. N. Y. Ent. Soc. 9: 51.

Eucosma perplexana Fernald, in Dyar, 1902, List N. Amer. Lepidop., no. 5130; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 6983.

Epinotia perplexana Heinrich, 1923, U. S. Natl. Mus. Bul. 123: 202-203.

Six adults of this species emerged October 16 to October 30, 1923, from mature pods of a wild cowpea, *Vigna repens* (Linnaeus) Kuntze, collected on the north shore of Galveston Bay at Smith Point October 3, 1923. The larvae fed upon the seeds and pupated within the pods.

SUBFAMILY OLETHREUTINAE

OLETHREUTES MALACHITANA (ZELLER)

Grapholitha malachitana Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 292-293.

Olethreutes malachitana Dyar, 1902, List N. Amer. Lepidop., no. 5044.

A large series of moths was reared during October, 1922, from larvae collected in folded leaves of young persimmon trees (*Diospyros virginiana* Linnaeus), at Lake Charlotte September 22, 1922. One specimen of a parasitic dipteran, *Lixophaga variabilis* Coquillett, was reared September 26 from this material.

¹⁰ HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 822, illus. 1921.

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WATER AND WATER SOLUTIONS OF ORGANIC COMPOUNDS AS DIPS FOR THE SOIL OF POTTED PLANTS INFESTED WITH THE JAPANESE BEETLE¹

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INTRODUCTION

The soil about the roots of plants grown in the open within the area infested by the Japanese beetle, *Popillia japonica* Newm., sometimes contains larvae of this insect (8, 9, 17, 18, 20),² yet large numbers of plants are grown within the infested area for commercial purposes under outdoor conditions. A Federal quarantine prohibits the shipment of such plants to points outside the infested zone unless the absence of the insect in the products in question is assured. Investigations were therefore undertaken at the Japanese Beetle Laboratory to develop methods for destroying the insect in the soil, with the object of applying these methods, wherever possible, to the treatment of the various types of plants grown commercially within the restricted area (6, 7, 10, 11, 12, 13, 14). Certain of the methods developed have been used successfully in the treatment of potted plants; for instance, the infestation of some varieties of plants growing in small pots has been prevented by mixing lead arsenate with the soil, and established infestations have been destroyed in tubs and the larger pots of ferns and other plants by percolating a dilute carbon disulphide emulsion through the soil. The treatment of pots smaller than 4 inches in diameter by the percolation method has not, however, been successful on account of the mechanical difficulties encountered and the cost of individual treatments of small pots in large quantities. The fumigation method (13), used so successfully with larger plants, could not be employed with smaller ones because of the mechanical difficulties. The experiments reported in this paper were made to determine whether it was economically possible to destroy an established infestation in the soil of several thousand small potted plants by dipping the pots in water or in toxic solutions.

WATER AS A DIP FOR SOIL INFESTED WITH LARVAE

It had been observed in the course of other related work that larvae were apparently uninjured by submersion in water for a period of 30 hours, but the maximum length of time that a larva could live under water was not known. Since water is so readily available for dipping purposes, it was deemed advisable to determine the period of submersion necessary to asphyxiate the larvae.

¹ Received for publication June 7, 1926; issued November, 1926. Contribution No. 9 of the Japanese Beetle Laboratory, Bureau of Entomology, Riverton, N. J., in cooperation with the Agricultural Departments of New Jersey, Pennsylvania, and Delaware.

² Reference is made by number (italic) to "Literature cited," p. 827.

The period of submersion lethal to the larvae was determined by placing 100 larvae, without soil, in individual wire cages submerged in water and removing 5 larvae on each subsequent day for examination. It was found that the larvae resisted asphyxiation by water for 15 days.

FACTORS INFLUENCING THE RESISTANCE OF LARVAE TO ASPHYXIATION

In the course of this experiment, it was observed that the larvae remained inactive when submerged in water and were surrounded to some extent by a film of air. It was thought probable that this trapped air assisted in prolonging the life of the larva, since it was possible that the air in the film was maintained at a sufficient oxygen tension to continue the larval metabolism. The experiment with water was therefore continued to determine whether the concentration of dissolved oxygen in the water affected the rate of mortality of the submerged larvae.

The influence of the concentration of the dissolved oxygen in the water was studied by sealing larvae in bottles of water of known oxygen content and at various intervals determining the larval mortality and the quantity of dissolved oxygen. One series of fifty 130 c. c. bottles was completely filled with aerated sterile distilled water containing 0.9 mgm. of dissolved oxygen per 50 c. c., and a second series of an equal number of bottles was filled with boiled sterile distilled water having 0.6 mgm. of dissolved oxygen. Five larvae were submerged in the water of each bottle. The bottles were then sealed by means of rubber stoppers and paraffin and kept at a temperature of 70° F. On certain subsequent days two bottles of water containing initially 0.9 mgm. of dissolved oxygen and two bottles containing initially 0.6 mgm. were opened to determine the effect on the larvae³ and the oxygen content of the water.⁴

The mortality of the larvae, as shown in Table 1, was practically the same in the water containing 0.9 mgm. of dissolved oxygen as in that containing 0.6 mgm. Submersion for 15 days was necessary to kill the most resistant individuals in both concentrations of oxygen. The results indicate that there is no correlation between the initial quantity of dissolved oxygen in the water and the rate of mortality of the submerged larvae.

³ The larvae removed for observation were placed on the surface of moist soil. If a larva became active and burrowed into the soil within 48 hours subsequent to its removal from the water, it was considered as unaffected by the submersion; if it showed signs of life but failed to go normally into the soil, it was regarded as injured by the treatment; and if it exhibited no evidence of life, it was held under observation until the first indication of tissue disintegration before it was pronounced dead.

⁴ The method used for determining the dissolved oxygen in the water was a modification of that recommended by the Association of Official Agricultural Chemists for the determination of dissolved oxygen in potable water (*l. p. 89*). The procedure is briefly as follows: Before removing the larvae from a bottle, two 50 c. c. samples of water were withdrawn by means of a 50 c. c. pipette and discharged into 250 c. c. Erlenmeyer flasks, the delivery end of the pipette being placed on the bottom of the flasks to preclude the incorporation of atmospheric oxygen in the flowing water. Two c. c. of manganous sulphate solution and 2 c. c. of sodium hydroxide-potassium iodide solution were added below the surface of the water by means of pipettes and mixed by gentle whirling to avoid undue agitation of the surface of the water. Sodium hydroxide reacts with manganous sulphate to form manganous hydroxide ($Mn(OH)_2$) which, having a great affinity for oxygen, unites with the dissolved oxygen in the water to form manganous acid (H_2MnO_3). This acid in contact with alkaline manganous hydroxide is converted into a brown flocculent precipitate of the manganous manganites ($Mn.MnO_3$ or $Mn.H_2(MnO_3)_2$). Stoppers were placed in the flasks until the precipitate of the manganites had settled. Two c. c. of 50 per cent sulphuric acid was then added. The hydriodic acid, formed by the reaction of sulphuric acid with potassium iodide, decomposed the manganite precipitate with the liberation of free iodine in direct proportion to the quantity of dissolved oxygen originally present in the water. The contents of the flask were then titrated against N/40 sodium thio-sulphate, 1 c. c. of which was equivalent to 0.2 mgm. of dissolved oxygen at 0° C. and 760 mm. pressure, according to the usual iodometric procedure.

TABLE 1.—*Relation of dissolved oxygen in water to larval mortality*

Number of days larvae were submerged	Using boiled distilled water				Using aerated distilled water			
	Dissolved oxygen per 50 c. c. (average)	Larvae dead	Larvae injured	Larvae unaffected	Dissolved oxygen per 50 c. c. (average)	Larvae dead	Larvae injured	Larvae unaffected
	<i>Mgm.</i>				<i>Mgm.</i>			
0.....	0.55	0	0	5	0.89	0	0	5
	.60	0	0	5	.92	0	0	5
7.....	.53	0	0	5	.44	0	0	5
	.40	1	0	4	.41	0	0	5
9.....	.38	2	0	3	.47	1	0	4
	.44	1	0	4	.51	0	0	5
12.....	.38	1	0	4	.48	1	0	4
	.39	1	0	4	.47	1	0	4
13.....	.20	3	0	2	.34	1	0	4
	.27	1	0	4	.29	0	0	5
14.....	.16	5	0	0	.17	4	1	0
	.18	4	1	0	.20	5	0	0
15.....	.17	5	0	0	.20	5	0	0
	.20	5	0	0	.11	5	0	0
16.....	.17	5	0	0	.20	5	0	0
	.17	5	0	0	.20	5	0	0

This resistance of *Popillia* larvae to asphyxiation is not unusual among insects. It has been recognized for a number of years that insects are difficult to kill by suffocation. As early as 1670, Boyle (3, *p.* 2051-2054) confined grasshoppers, caterpillars, beetles, and flies in the best vacuum which he could obtain and found that the insects recovered when returned to air. Treviranus (19, *p.* 151) observed great variation among insects in their oxygen requirements. Bourcart (2, *p.* 30) states that it was necessary to submerge a field infested with soil insects for a relatively long period, 8 to 60 days, in order to asphyxiate them. Shafer (16) more recently found that insects could survive submersion in atmospheres of carbon dioxide and hydrogen for several hours.

When the results of the experiments with the *Popillia* larvae are considered from the standpoint of their practical application, it is seen that the necessary period of submersion is too prolonged to be employed. Submersion in water is therefore of little apparent value as a means of destroying the larvae in the soil of small potted plants. In view of these results, a series of experiments was begun to determine the value of water solutions of organic compounds as dips for the small potted plants infested with larvae.

COMPARATIVE VALUE OF WATER SOLUTIONS OF ORGANIC COMPOUNDS AS DIPS FOR INFESTED SOIL

Previous experiments (5, 7, 10, 12, 14) have shown that the larvae of the Japanese beetle are killed when removed from soil and submerged in water solutions or emulsions of such compounds as sodium cyanide, thymol, carbon disulphide, o-cresol, phenol, benzyl chloride, nitrobenzene, naphthalene, and others. Certain of these toxic solutions, prepared according to a procedure previously employed by the writer (7) were tested to determine their relative effectiveness in killing submerged larvae embedded in small masses of soil.

The results obtained by the method of experimentation necessarily chosen in classifying the few selected compounds are not conclusive,

but only indicate the compound worthy of more exhaustive tests. Ten liters of each dipping solution and 10 liters of tap water were prepared and held in suitable containers at a temperature of approximately 70° F. Eight of the previously infested 2-inch flowerpots of soil were submerged in each solution.⁵ At time intervals of 6, 12, 24, and 30 hours two pots were taken from each solution to observe its effect and that of the period of submersion on the larvae. The larvae were removed from the treated soil as soon as it was withdrawn from the dipping solutions and were placed in pots of untreated soil for subsequent examination.

The results of these dipping tests, which were obtained 72 hours after the last pot was removed from the solutions, are summarized in Table 2. Carbon disulphide emulsion was the most effective of the compounds penetrating in lethal concentration into the infested soil.

TABLE 2.—*Ability of dilute organic emulsions to penetrate into submerged pots of soil and kill the larvae contained therein*

Test No.	Toxic agent of the emulsion	Concentration of toxic agent	Effect on larvae submerged at 70° F.							
			6 hours		12 hours		24 hours		30 hours	
			Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
		<i>Gm. per liter</i>								
1-8.....	o-Cresol ($\text{CH}_3\text{C}_6\text{H}_4\text{OH}$).....	2.0	2	0	2	0	1	1	2	0
			2	0	1	1	1	1	0	2
9-16.....	do.....	1.0	2	0	1	1	1	1	2	0
			2	0	2	0	1	1	0	2
17-24.....	Phenol ($\text{C}_6\text{H}_5\text{OH}$).....	2.0	2	0	2	0	1	1	1	1
			1	1	1	1	1	1	1	1
25-32.....	do.....	1.0	1	1	2	0	1	1	1	1
			1	1	1	1	1	1	1	1
33-40.....	Benzyl chloride ($\text{C}_6\text{H}_5\text{CH}_2\text{Cl}$).....	3.5	2	0	2	0	2	0	2	0
			2	0	2	0	1	1	2	0
41-48.....	do.....	2.0	1	1	1	1	1	1	1	1
			1	1	2	0	1	1	2	0
49-56.....	Carbon disulphide (CS_2).....	2.0	2	0	2	0	2	0	2	0
			2	0	2	0	2	0	2	0
57-64.....	do.....	1.0	2	0	2	0	2	0	2	0
			2	0	2	0	2	0	2	0
65-72.....	Nitrobenzene ($\text{C}_6\text{H}_5\text{NO}_2$).....	3.5	2	0	2	0	2	0	0	2
			2	0	2	0	2	0	2	0
73-80.....	Sodium cyanide (NaCN).....	2.0	2	0	2	0	2	0	2	0
			2	0	2	0	2	0	2	0
81-88.....	do.....	1.0	2	0	2	0	1	1	2	0
			2	0	2	0	2	0	1	1
89-96.....	Water only.....		0	2	1	1	2	0	0	2
			1	1	1	1	0	2	0	2
97-99.....	Control (soil not submerged).....								0	2
									0	2

CARBON DISULPHIDE EMULSION AS A DIP FOR SOIL INFESTED WITH LARVAE

The previous experiments indicated that dilute carbon disulphide emulsion might be effective as a dip in destroying the infestation in such small masses of soil as are contained in 2-inch and in 4-inch

⁵ Three weeks prior to the actual treatment, 70 gm. of rich, moist greenhouse soil was placed in each of 100 2-inch flowerpots and infested with two third-instar larvae. The pots were maintained at the customary greenhouse temperature, 50° to 70° F., and sprinkled daily with water. As a result, the soil was compact and the larvae in a normal active state when the pots were submerged in the toxic solutions.

flowerpots. It was determined therefore to test further the efficiency of this emulsion by treating a large number of salvia and nasturtium plants growing in 2-inch pots and pine and arbor vitae growing in 4-inch pots.

EFFICIENCY OF EMULSION WITH 2-INCH POTS

Emulsions were prepared for each series of plants in progressive dosages, namely, 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875, 1.000, 1.125, 1.250, and 1.375 c. c. of carbon disulphide per liter of solution. Ten liters of each concentration of the emulsion and 10 liters of untreated water, serving as a control, were placed in suitable containers and held at a temperature of approximately 70° F. throughout the submersion period.

Ten uniform plants of each series of salvia were selected, weighed, and submerged in each concentration of the carbon disulphide and in the water control so that the surface of the soil was just below the surface of the liquid. In the case of the nasturtiums, a certain proportion of which had failed to grow, it was necessary to reduce the number of plants in each solution from 10 to 5. When the submersion had been prolonged for 18 hours, the pots were removed from the solutions, freed of excess water, and reweighed. They were then returned to the greenhouse for subsequent observation to determine the indirect effect of the various concentrations on the aerial portions of the plants.

After a period of 4 to 6 weeks the aerial portions of the plants usually showed signs of stimulation, or injury, or were dead. The ball of soil about the roots of each plant was then carefully removed from the pot and crumbled to find the larvae. If a larva had been killed, it was often difficult to locate even a fragment of its body; but if it was unaffected or had been only slightly injured, it was usually very active and easily found.

The results of these tests, recorded in Table 3, indicate that there is practically no margin between the dosage of carbon disulphide in the dipping solution lethal to the larva and that injurious to the plant. Complete larval mortality was secured in series 1 with a dosage of 1.0 c. c. of carbon disulphide per liter; only 0.75 c. c. was required in series 2, whereas 1.375 c. c. was not sufficient in series 3. A dosage of 1.25 c. c., however, was fatal to salvia and nasturtium. This wide variation in the concentration necessary to kill the larvae is probably caused by the difference in the individual activity and location of the insects in the mass of soil. Larvae which are active or which are in the layers of soil in close proximity to the sides of the flowerpot are more readily killed than those which have burrowed into the center of the soil mass.

EFFICIENCY OF EMULSION WITH 4-INCH POTS

Although the results with the 2-inch potted plants were negative, it was deemed advisable to repeat the experiments with 4-inch pots. Pine and arbor vitae, growing in 4-inch pots of infested soil, were therefore subjected to the same treatment as that received by the plants in the 2-inch pots. The results of these tests were also negative.

TABLE 3.—Carbon disulphide emulsion as an insecticidal dip for soil of plants in 2-inch pots submerged for 18 hours at 70° F.

Series No.	Pot No.	Concentration of CS ₂	Solution imbibed by soil			Effect on larvae			Species of plant	Effect on plants
			Minimum in 10 pots	Maximum in 10 pots	Average in 10 pots	Number dead in 10 pots	Number alive in 10 pots	Percent dead in 10 pots		
		<i>C. c. per liter</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Per cent</i>					
1	1-10	0.125	0.2	10.4	2.58	0	20	0	Salvia	Stimulated.
	11-20	.250	1.9	8.3	2.48	0	20	0	do	Do.
	21-30	.375	2.1	13.6	3.28	0	20	0	do	Do.
	31-40	.500	1.1	7.7	2.40	4	16	20	do	Do.
	41-50	.625	1.0	11.6	3.30	6	14	30	do	Unaffected.
	51-60	.750	4.5	11.2	4.37	8	12	40	do	Sickly.
	61-70	.875	1.5	8.7	2.45	19	1	95	do	40 per cent dead.
	71-80	1.000	1.4	15.3	3.51	20	0	100	do	70 per cent dead.
	81-90	1.125	1.2	10.0	2.53	20	0	100	do	80 per cent dead.
	91-100	1.250	2.8	13.2	3.57	20	0	100	do	100 per cent dead.
2	101-110	1.375	2.5	10.0	3.92	20	0	100	do	Do.
	111-120	(¹)	.7	16.3	3.76	1	19	5	do	Unaffected.
	121-125	.125	2.8	10.6	4.19	0	10	0	Nasturtium	Do.
	126-130	.250	8.1	37.3	11.40	3	7	30	do	Do.
	131-135	.375	11.7	35.4	7.70	1	9	10	do	Do.
	136-140	.500	11.1	15.8	7.52	8	2	80	do	Do.
	141-145	.625	11.5	27.2	9.42	8	2	80	do	Do.
	146-150	.750	19.1	29.9	12.72	10	0	100	do	Do.
	151-155	.875	14.2	31.1	10.55	10	0	100	do	Sickly.
	156-160	1.000	15.0	25.2	8.23	10	0	100	do	20 per cent dead.
3	161-165	1.125	10.3	21.0	7.73	10	0	100	do	80 per cent dead.
	166-170	1.250	12.2	29.9	9.13	10	0	100	do	100 per cent dead.
	171-175	1.375	11.0	21.0	9.04	10	0	100	do	Do.
	176-180	(¹)	11.0	16.0	6.43	0	10	0	do	Unaffected.
	181-190	.125	8.7	14.5	6.42	0	20	0	Salvia	Stimulated.
	191-200	.250	10.0	29.4	10.66	0	20	0	do	Do.
	201-210	.375	10.0	25.0	8.65	5	15	25	do	Do.
	211-220	.500	11.0	28.0	10.00	9	11	45	do	Unaffected.
	221-230	.625	13.0	25.0	10.28	4	16	20	do	20 per cent sickly.
	231-240	.750	40.0	19.0	6.52	6	14	30	do	60 per cent sickly.
3	241-250	.875	5.0	22.0	8.71	8	12	40	do	Do.
	251-260	1.000	5.0	29.0	9.36	11	9	55	do	90 per cent dead.
	261-270	1.125	18.0	26.0	13.11	19	1	95	do	100 per cent dead.
	271-280	1.250	12.0	25.0	9.65	14	6	70	do	Do.
	281-290	1.375	5.0	20.0	6.04	12	8	60	do	Do.
	291-300	(¹)	7.0	26.0	9.34	0	20	0	do	Unaffected.

¹ None (control).

DISCUSSION OF SOIL CONDITIONS LIMITING THE EFFECTIVENESS OF CARBON DISULPHIDE EMULSION IN SUBMERGED SOIL

The larvicidal action of a dilute carbon-disulphide emulsion under these conditions is dependent upon the volume of the solution penetrating into the soil and upon the length of time during which the active principle of the solution maintains its potency in contact with the soil. The pore space is occupied by water and air. If the water content of the soil is low the air space is large. Under optimum conditions for growing plants, over half of the pore space is filled with water. It is apparent therefore that the entire volume of solution imbibed by the soil from even an unlimited source would necessarily be limited.

The toxic solution which penetrates into the soil is markedly affected by the soil particles. It is well known that dissolved as well as suspended material can be more or less completely removed from water by passing the solution through sand or soil. This removal of the solute or suspended material from water is the result of absorption, the chemical reaction between the soil and the dissolved substance, and of adsorption, the condensation or concentration of the dissolved substance on or about the surface of the soil particles (4, p. 59-69; 15, p. 349-374). The dissolved or suspended substance is not uniformly distributed throughout the soil by the water and tends to become concentrated in the outer layers of the soil mass. Even in the concentrated area as well as in other parts of the soil mass a certain portion of the toxic solute is rendered impotent by being absorbed in the moisture film surrounding the soil particles. The diffusion of the toxic agent into the soil to replace that portion of the imbibed solution removed by adsorption is a slow process and is of little value in killing larvae. The results obtained by Leach and Thomson (10) with infested soil dipped in toxic solutions gave every indication that the portion of the initially imbibed solution which was unaffected by absorption or adsorption was responsible for the insect mortality. The effective action of the carbon-disulphide emulsion is dependent therefore upon the initial concentration obtained throughout the soil mass.

It is apparent from the brief discussion of the soil conditions which influence the insecticidal efficiency of the carbon-disulphide emulsion in the submerged soil, that the action of a toxic dipping solution is uncertain and almost entirely dependent upon favorable soil conditions. The extreme variability in these conditions would make rather dubious the general use of any dipping solution.

SUMMARY

Experiments were made to determine whether an established infestation of the Japanese beetle in the soil of small potted plants could be destroyed by submersion for a definite period in water or in water solutions (emulsions) of o-cresol, phenol, benzyl chloride, carbon disulphide, nitrobenzene, and sodium cyanide.

The larvae resisted asphyxiation in water for 15 days. No correlation was found between the rate of larval mortality and the concentration of the dissolved oxygen of the water.

Carbon-disulphide emulsion was the most effective of the solutions in killing the larvae in the submerged soil. The insecticidal concentrations, however, usually injured plants.

It was apparent in these experiments that the effective action of any dipping solution is so dependent upon soil imbibition, soil adsorption, and soil absorption as to limit the application of even an effective insecticide to conditions where these factors are favorable.

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NUTRITIVE VALUE OF PROTEIN IN BEEF EXTRACT, OX BLOOD, OX PALATES, CALF LUNGS, HOG SNOOTS, AND CRACKLINGS¹

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INTRODUCTION

The purpose of the investigation herein reported was to determine the nutritive value of the protein in beef extract, ox blood, ox palates, calf lungs, hog snouts, and cracklings, as measured by the growth induced in albino rats. In these experiments, as in previous studies with other meat products (4, 5),³ the term "protein" is used in a general sense to include all organic nitrogenous compounds, whether true proteins or not. In most meat products only a small proportion of the nitrogen is a constituent of nonproteins, but in beef extract at least one-half of the nitrogen is in that form.

DESCRIPTION OF PRODUCTS

In the United States beef extract is an important product of the meat-canning industry. It is prepared by concentrating in vacuum kettles the clear broth obtained from cooking fresh beef. Two types of extract are prepared, depending on the degree to which the beef broth has been concentrated, viz, fluid extract, which contains approximately 50 per cent moisture, and solid extract, which contains approximately 25 per cent moisture.

In addition to beef extract, similar extracts are prepared from various edible parts and organs of cattle, sheep, and hogs, and from the broth obtained from cooking corned beef preparatory to canning. These extracts must be labeled to show their true character, and the term "beef extract" is restricted to that product which has been prepared entirely from fresh beef.

Blood is an important potential source of food for man and animals. As collected in the packing house it is equal to approximately 10 per cent of the dressed weight of fat cattle, 8 per cent of the weight of sheep, and 4 per cent of the weight of hogs. On account of a natural distaste for blood as a food, however, it is used to a very limited extent in the human diet, chiefly as an ingredient of sausage, but an appreciable quantity of the dried product is fed to farm animals. Only blood from cattle is used as human food.

Ox palates, commonly called "hard palates," comprise the tissues in the roofs of the mouths of cattle. This product consists largely of rather tough connective tissue, together with some muscle and fatty tissue. Ox palates are first cooked and then utilized in the manufacture of sausage. They are used only to a limited extent for human food.

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³ Reference is made by number (italic) to "Literature cited," p. 842.

Calf lungs, as well as those of cattle and sheep, find limited use in the human dietary.

Cracklings are the residue from the rendering of lard or beef fat in open kettles. They consist largely of connective tissue, together with a small proportion of muscle and variable quantities of fat. This product is used to a limited extent as human food, the bulk of it being utilized either for feeding farm animals or as a constituent of fertilizers.

Hog snouts, which comprise the facial tissues on the upper jaw extending from the eyes to the tip of the nose, consist almost entirely of connective tissue and fat. This product is used chiefly in the preparation of potted products, sausage, and similar articles

PREVIOUS INVESTIGATIONS

BEEF EXTRACT

Beef extract has been the subject of numerous investigations, but most of these have related to its physiological effects, and relatively little work has been done to determine the nutritive value of the nitrogenous compounds in this product. Although approximately one-half of the nitrogen in beef extract is present as a constituent of meat bases which have no food value, the remainder is found chiefly in the form of proteoses, peptones, amino acids, and gelatin; compounds which might be expected to have considerable food value.

Thompson (10) fed dog biscuit to dogs until they remained constant in weight. Beef extract was then added to the diet. The dogs increased in weight after the addition of the beef extract, but returned to their former weights when the extract was omitted. A part of the nitrogen in the beef extract was retained by the dogs, but the most of it was excreted in the urine. The beef extract induced a more complete digestion of the dog biscuit, as evidenced by a reduction in the quantity and the nitrogen content of the feces.

Thompson, Caldwell, and Wallace (11), in experiments with human subjects and with dogs, found that the addition of beef extract to the diet caused an increase in weight and the retention by the body of a considerable proportion of the nitrogen in the extract. There was also a decreased excretion of nitrogen in the feces, due apparently to the better digestion of the ordinary diet. These writers concluded, therefore, that beef extract has both a direct and an indirect nutritive value.

Hutchison (6, *p. 94, 95*) states that "rats which were fed on 4 grammes of meat extract daily died quite as soon as other rats which got no food at all." He states that "[beef] extract, though containing too little protein matter to be in itself a food, may act as a valuable adjuvant to other foods, especially where appetite and digestion are feeble."

BLOOD

A considerable number of feeding experiments with blood have been conducted with farm animals at the agricultural experiment stations in this country and in Canada, but it does not seem desirable to discuss these in detail. It will suffice to quote briefly from a recent text by Henry and Morrison (3, *p. 186, 309, 375, 652*) regarding the value of blood as a food for farm animals.

Dried blood is particularly useful with young pigs and calves, as a skim milk substitute, or for sickly animals. Its usual high price stands in the way of its common use for other animals, but it has been fed with success to horses, dairy cows, and sheep.

Tankage and blood meal were found by Burkett at the North Carolina station to be useful for run-down, thin horses, 1 to 2 lbs. of tankage or 1 lb. of blood meal being employed. Pott states that blood meal has given excellent results in horse feeding. As such animal by-products are unpalatable to horses, they must be mixed with well-liked feeds.

Blood meal is not commonly used for feeding dairy cows, primarily due to its high price, but it is quite extensively used in calf feeding. It is occasionally used in stimulating cows to their utmost when on test, but only 1 to 2 lbs. should be added to the ration. It is not palatable to cattle, though they can be taught to eat it when mixed with other feeds. In a trial by Lindsey at the Massachusetts station 1.1 lbs. blood meal was equal to 2.2 lbs. cottonseed meal.

Blood meal is used but relatively little for swine feeding, except sometimes for very young pigs, as tankage is usually a more economical source of protein.

Imabuchi (7) determined the digestibility of dried blood in a test with a dog. The animal was first fed a ration consisting of 300 gm. of horse meat and 50 gm. of fat meat for a period of 5 days; then a ration composed of 46 gm. of dried blood, 100 gm. of horse meat, and 50 gm. of fat meat for a period of 10 days, and finally the first-named ration for a period of 5 days. The digestibility of the protein in the rations fed during the three periods was as follows: Period 1, 94.3 per cent; period 2, 86.6 per cent; and period 3, 94 per cent. The dog was in nitrogen equilibrium during the first period but lost 3.3 gm. of nitrogen, corresponding to 117 gm. of flesh, during the second period when blood was included in the ration.

Blum (1) states that blood serum is utilized practically as completely as meat, but that the red corpuscles have a much lower value, the protein in the latter being only 70 to 75 per cent digestible. Whole blood is considered to have a somewhat lower nutritive value than meat.

Opel (9, *p.* 141-147) states that the protein in ox blood was found by Salkowski to be 95 per cent digestible and that the protein in sheep blood was 96.5 per cent digestible, as determined by Beck, the experiments being conducted with dogs. Opel states also that serum protein is digested as thoroughly as that in meat, but that the protein in the corpuscles is not so completely digested, 25 to 30 per cent of the nitrogen appearing in the feces.

Hutchinson (6, *p.* 72, 73) states that two French experimenters found that 2 pounds daily of blood was not sufficient to maintain life in dogs for more than a month. Hemoglobin, which makes up the larger part of the protein in blood, is reported to be not well utilized.

OX PALATES

The digestibility of ox palates was determined by Langworthy and Holmes (8). The palates were first cooked and then fed to men as the chief source of protein in a mixed diet. The digestibility of the total protein in the diet was found to be 87.3 per cent, and that in the ox palates alone was calculated as being approximately 86.8 per cent. It is stated that the protein of cooked hard palates is somewhat less thoroughly digested than that of the common cuts of meat.

OTHER PRODUCTS

Although no experimental evidence concerning the nutritive value of the protein in calf lungs, hog snouts, or cracklings was found in the

literature, the following statements indicate the apparent nutritive value of these products:

Hutchison (6, *p.* 73) states that lungs "are sometimes eaten, but can not be regarded as a really good form of food." They are described as being largely composed of an elastic material, an albuminoid, and as being imperfectly digested and useless as building material in the body.

Pork cracklings, according to Henry and Morrison (3, *p.* 186) are fully as valuable as tankage for swine. Bull (2, *p.* 219) states that cracklings are considered more digestible than tankage and that the two products have about the same value as feed for hogs. He also recommends cracklings as a feed for poultry.

EXPERIMENTAL PROCEDURE

METHODS

The methods employed in this investigation are essentially the same as those followed by the writers in previous studies with other meat products (4, 5). Each meat product under examination constituted the only source of nitrogen in a ration which also contained adequate quantities of vitamins A, B, and D and of mineral matter. As a rule, each ration was fed to six young, male, albino rats weighing approximately 40 gm. each at the start, but in a few instances mature rats of both sexes were used. Except where indicated on the graphs, the curves denote the growth made by male rats.

DESCRIPTION OF SAMPLES

The beef extract, which was of the solid or paste variety, was obtained direct from a factory. The extract was dissolved in water, mixed with starch, and the mixture was dried and ground so as to facilitate incorporation in the ration.

Defibrinated ox blood was dried in the laboratory in a current of air at a temperature under 70° C. All the dried product was found to be practically soluble in water. A portion of the dried blood was heated 30 minutes in an autoclave under 15 pounds' steam pressure.

The dried serum and hemoglobin were obtained from a factory which prepares these products on a commercial scale. The serum was prepared so as to eliminate fibrin.

The ox palates were first cooked, then ground, dried in a current of air at 60° to 70° C., and again ground; the calf lungs were prepared in a similar manner except that they were not cooked.

The hog snouts were ground, heated on a steam bath to remove most of the fat, and the tissue residue was dried in a current of air and then ground fine.

Two lots of pork cracklings, each of which contained only a little muscle tissue, were obtained from different packing houses. The cracklings were ground fine before use in the rations.

COMPOSITION OF MEAT PRODUCTS

The proximate composition of the samples of meat products is reported in Table 1. The percentage of crude protein was obtained by multiplying the percentage of nitrogen by 6.25. These results do not necessarily indicate the true protein content of each product, certainly not of the beef extract, but they offer a convenient basis for

comparing the nutritive values of the mixed nitrogenous compounds in the several products.

TABLE 1.—Composition of dried meat products ^a

Laboratory No.	Product	Moisture	Ash	Nitrogen	Crude protein (nitrogen $\times 6.25$)	Fat
		Per cent	Per cent	Per cent	Per cent	Per cent
1256	Beef extract ^b	14.32	21.75	11.27	70.44	—
1186	Ox blood	—	—	14.40	90.00	0.79
1232	Ox blood (heated in autoclave)	—	—	14.59	91.19	—
1214	Serum	10.01	13.57	10.75	67.19	.34
1237	Serum	—	13.35	11.59	72.43	—
1213	Hemoglobin	15.12	1.35	13.28	83.00	.34
1255	Ox palates	—	—	10.05	62.81	32.99
1287	Calf lungs	—	—	12.11	75.69	6.93
1258	Hog snouts	—	—	14.01	87.56	7.65
1269	Cracklings	—	—	13.86	86.62	3.48
1284	Cracklings	—	—	14.35	89.69	6.79

^a The chief object of these analyses was to determine the nitrogen and fat; in some cases the moisture and ash were determined also.

^b The beef extract was not dried.

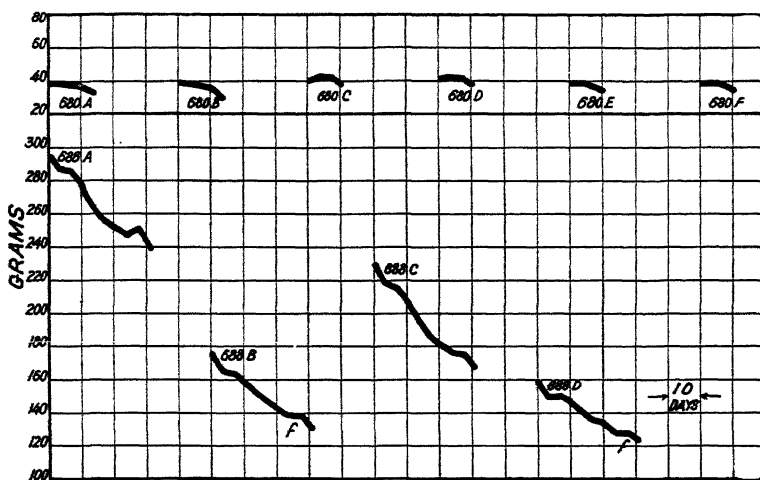


FIG. 1.—Growth curves of rats fed a ration containing 18 per cent of crude protein (nitrogen $\times 6.25$) from beef extract

RESULTS OF FEEDING EXPERIMENTS

BEEF EXTRACT

A ration containing 18 per cent of crude protein (nitrogen $\times 6.25$) from beef extract, but which probably contained less than 9 per cent of true protein, was fed to 6 young male rats and to 4 mature ones. The results of these tests are reported in Figure 1 and Table 2, the two groups of rats being numbered 680 and 688, respectively. From Figure 1 it is evident that the ration was very inadequate, even for maintenance, for either group of rats. It was possible to continue the experiment with the mature rats for a period of 31 days, but each rat lost weight steadily throughout the test, the losses ranging from 19.5 to 27.4 per cent. Food consumption was approximately normal for the rats in each group.

In this connection it may be stated that a ration containing 5 per cent of protein of good quality and 10 per cent of fat is adequate for maintenance in rats, whereas 10 per cent of good protein will induce approximately normal growth.

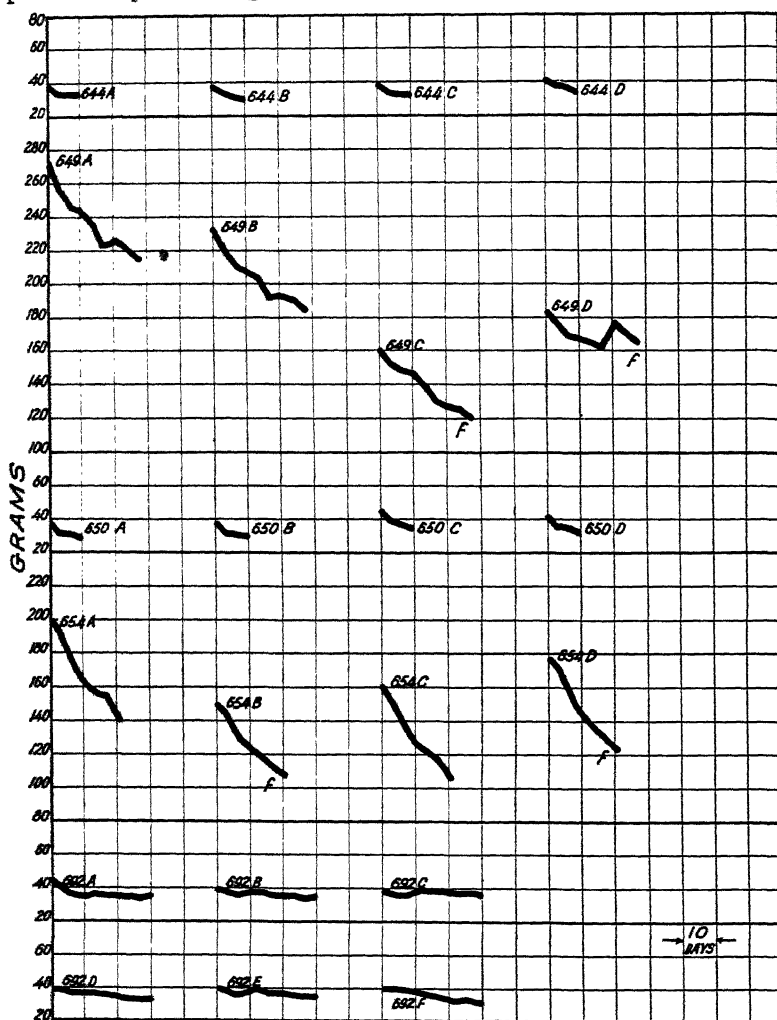


FIG. 2.—Growth curves of rats fed rations containing dried ox blood as the source of protein. Rats numbered 644 and 649, respectively, received 20 per cent protein; rats numbered 650 and 654, respectively, 50 per cent protein; and rats numbered 692, 15 per cent protein.

OX BLOOD

The results of the feeding tests with ox blood are reported in Figure 2 and in Table 2. Four young male rats, numbered 644, were fed a ration containing 20 per cent of crude protein from dried, defibrinated blood which had been heated in an autoclave after drying. It is apparent that the ration was inadequate even for maintenance (fig. 2), but on referring to Table 2 it will be noted that, with one exception, the food consumption of the rats was very

low. The average daily food intake for each of three rats ranged from 1.7 to 1.9 gm.—quantities far below their normal requirements—but the average daily food intake of the fourth rat, 3.9 gm. was practically normal.

The same ration was fed to four mature rats numbered 649 (fig. 2 and Table 2), and it will be noted that they lost weight more rapidly than the young rats. Although the ration contained adequate quantities of vitamins A, B, and D, each rat was fed daily four drops of cod-liver oil. From Table 2 it may be noted that the food consumption of these rats also was below normal.

A ration containing 50 per cent of protein from dried raw blood was fed to young male rats numbered 650 (fig. 2 and Table 2). Each rat received 4 drops of cod-liver oil daily in addition to 2 per cent of the oil already present in the ration. All of these rats lost weight throughout the test. The same ration was fed to 4 mature rats numbered 654 (fig. 2 and Table 2), but the loss in weight was more pronounced than in the young rats. The average daily food intake for the rats in each group was much below the maintenance requirement.

A ration containing 15 per cent of protein from dried raw blood was fed 6 young male rats numbered 692, and each rat was fed daily 0.1 gm. vitamin B from yeast and 5 drops of cod-liver oil in addition to the quantities of these vitamins already present in the ration. From Figure 2 it is evident that these rats nearly maintained their weight; but in Table 2 it will be noted that the food intake was low

TABLE 2.—Results of feeding tests with beef extract, ox blood, serum, and hemoglobin as sources of protein in the diet of rats

Source of protein	Protein in ra- tion	Rat No.	Duration of test	Initial weight	Final weight	Gain (+) or loss (—) in weight	Total feed con- sumed	Gain in weight per gram of protein consumed	
								30 days	60 days
	<i>Per cent</i>		<i>Days</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Beef extract.....	18	680 A	15	38	32	-6	53		
Do.....	18	680 B	15	39	30	-9	47		
Do.....	18	680 C	11	40	38	-2	44		
Do.....	18	680 D	11	41	37	-4	41		
Do.....	18	680 E	11	39	34	-5	36		
Do.....	18	680 F	11	39	34	-5	35		
Do.....	18	688 A	31	297	239	-58	330		
Do.....	18	688 B	31	176	130	-46	183		
Do.....	18	688 C	31	230	167	-63	186		
Do.....	18	688 D	31	160	123	-37	153		
Ox blood (heated in auto- clave).....	20	644 A	10	38	34	-4	18		
Do.....	20	644 B	10	38	30	-10	17		
Do.....	20	644 C	10	39	33	-6	19		
Do.....	20	644 D	10	41	33	-8	39		
Do.....	20	649 A	28	274	215	-59	144		
Do.....	20	649 B	28	234	185	-49	139		
Do.....	20	649 C	28	161	121	-40	81		
Do.....	20	649 D	28	184	164	-20	139		
Ox blood.....	50	650 A	10	38	29	-9	16		
Do.....	50	650 B	10	38	30	-8	16		
Do.....	50	650 C	10	45	34	-9	19		
Do.....	50	650 D	10	42	32	-10	26		
Do.....	50	654 A	21	202	139	-63	61		
Do.....	50	654 B	21	150	107	-43	61		
Do.....	50	654 C	21	161	105	-56	35		
Do.....	50	654 D	21	178	122	-56	53		
Do.....	15	692 A	30	44	35	-9	45		
Do.....	15	692 B	30	39	34	-5	54		
Do.....	15	692 C	30	38	36	-2	56		
Do.....	15	692 D	30	39	32	-7	48		
Do.....	15	692 E	30	40	35	-5	48		
Do.....	15	692 F	30	40	31	-9	67		

TABLE 2.—Results of feeding rats with beef extract, ox blood, serum, and hemoglobin as sources of protein in the diet of rats—Continued

Source of protein	Protein in ration	Rat No.	Duration of test	Initial weight	Final weight	Gain (+) or loss (—) in weight	Total feed consumed	Gain in weight per gram of protein consumed	
								30 days	60 days
	Per cent		Days	Grams	Grams	Grams	Grams	Grams	Grams
Serum No. 1214.....	10	623 A	32	38	70	+32	133	2.11	-----
Do.....	10	623 B	42	38	58	+20	134	1.62	-----
Do.....	10	623 C	32	42	97	+55	214	2.71	-----
Do.....	10	623 D	32	40	65	+25	149	1.80	-----
Do.....	10	623 E	32	40	65	+25	133	2.02	-----
Do.....	10	623 F	32	38	72	+34	154	2.18	-----
Average gains.....						+32		2.07	-----
Serum No. 1235.....	10	656 A	30	43	46	+3	109	-----	-----
Do.....	10	656 B	30	40	39	-1	94	-----	-----
Do.....	10	656 C	28	40	45	+5	88	-----	-----
Do.....	10	656 D	21	45	49	+4	78	-----	-----
Serum No. 1214.....	15	637 A	62	45	100	+55	324	1.94	1.21
Do.....	15	637 B	62	38	140	+102	405	2.53	1.70
Do.....	15	637 C	62	41	173	+132	471	2.39	1.90
Do.....	15	637 D	62	40	122	+82	333	2.48	1.47
Do.....	15	637 E	62	40	138	+98	413	2.29	1.62
Do.....	15	637 F	62	39	166	+127	441	2.33	1.94
Average gains.....						+99		2.33	1.64
Hemoglobin.....	10	624 A	14	38	32	-6	35	-----	-----
Do.....	10	624 B	14	39	30	-9	38	-----	-----
Do.....	10	624 C	14	40	32	-8	36	-----	-----
Do.....	10	624 D	13	40	32	-8	31	-----	-----

OX SERUM

Six young rats numbered 623 (fig 3 and Table 2) were fed a ration containing 10 per cent of serum protein. The rats grew at a rate far below normal for rats getting a ration containing 10 per cent of protein of good quality, such as that in veal (fig. 4). The rats fed the serum protein made an average gain in weight of only 2.07 gm. for each gram of protein consumed, as compared with a gain of 2.86 gm. for the rats getting 10 per cent of veal protein (Table 3).

A ration containing 10 per cent of protein from another lot of serum from the same factory was fed to rats numbered 656 (fig. 3 and Table 2), but this ration was scarcely more than adequate for maintenance.

Rats numbered 637 (fig. 3 and Table 2) were fed a ration containing 15 per cent of protein from the same lot of serum as that fed to rats No. 623. This ration induced fair but not normal growth. These rats made an average gain in weight of 2.33 gm. for each gram of protein consumed during a 30-day period, as compared with a gain of 2.86 gm. for rats fed 10 per cent of veal protein during a like period (Table 3).

OX HEMOGLOBIN

ration containing 10 per cent of hemoglobin was fed to rats numbered 624 (fig. 3 and Table 2). This ration proved to be inadequate even for maintenance, although food consumption was not much below normal.

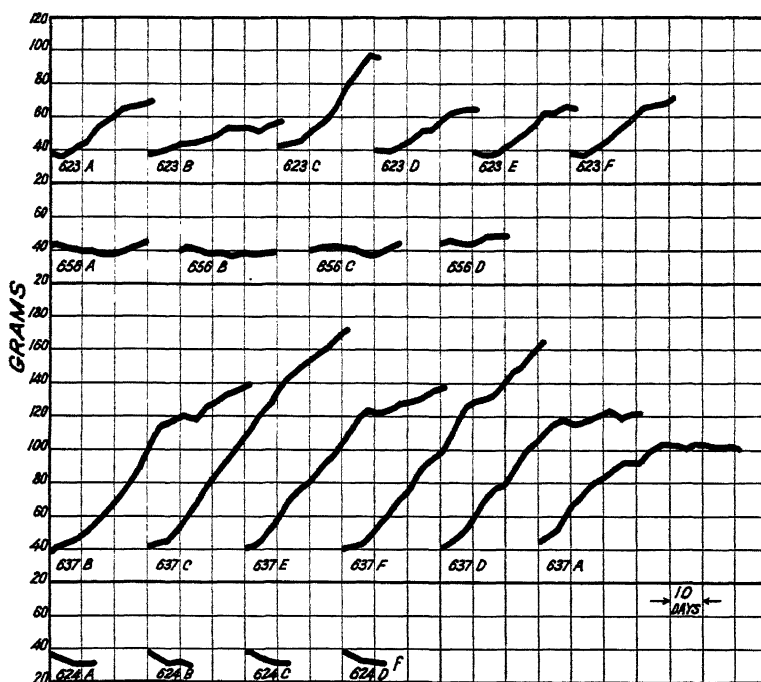


FIG. 3.—Growth curves of rats fed dried ox serum and hemoglobin as the sources of protein in the rations. Rats numbered 623 and 656, respectively, were fed 10 per cent of protein from two different lots of serums. Rats numbered 637 received 15 per cent of serum protein, and rats numbered 624 were fed 10 per cent hemoglobin

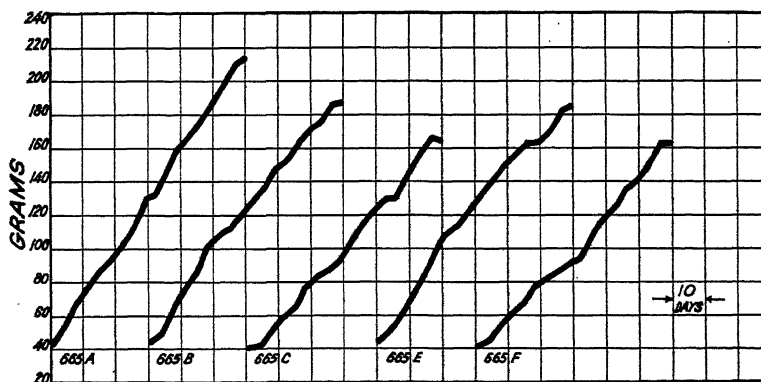


FIG. 4.—Growth curves of rats fed a ration containing 10 per cent of protein from veal

OX PALATES

Rations containing 10 and 15 per cent, respectively, of protein from ox palates were fed to rats numbered 678 and 679 (fig. 5 and Table 3). The rats getting 10 per cent of protein grew very slowly and those receiving 15 per cent made only slightly better growth. During the

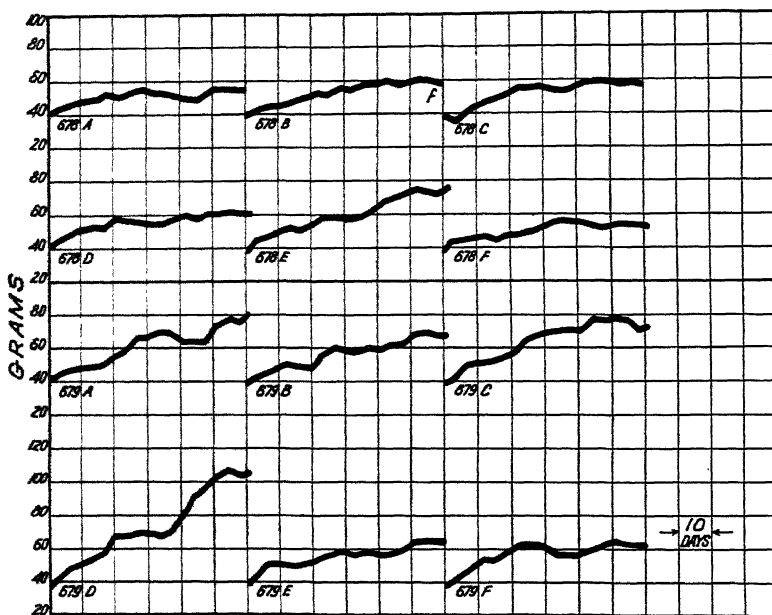


FIG. 5.—Growth curves of rats fed ox palates as the source of protein in their diet. Rats numbered 678 received 10 per cent, and rats numbered 679, 15 per cent protein

first 30 days of the test the rats receiving 10 per cent protein gained an average of only 1.22 gm. in weight for each gram of protein consumed, and those getting 15 per cent protein only 1.14 gm., as compared with a gain of 2.86 gm. for rats getting 10 per cent veal protein during a like period.

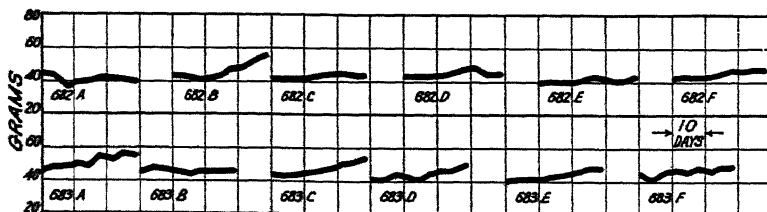


FIG. 6.—Growth curves of rats fed hog snouts as the source of protein in their diet. Rats numbered 682 received 10 per cent, and rats numbered 683, 15 per cent protein

HOG SNOOTS

Rats numbered 682 and 683, respectively, were fed rations containing 10 and 15 per cent of protein from hog snouts (fig. 6 and Table 3). The first group of rats made practically no growth, and the second group made only slight growth during a period of 29 days. Food consumption was approximately normal.

PORK CRACKLINGS

Two lots of cracklings were fed to rats numbered 697 and 731, respectively, the first group receiving 15 per cent protein, the second group 20 per cent (fig. 7 and Table 3). It is evident that these rations were only approximately adequate for maintenance, the first group making a slight average gain and the second group a slight average

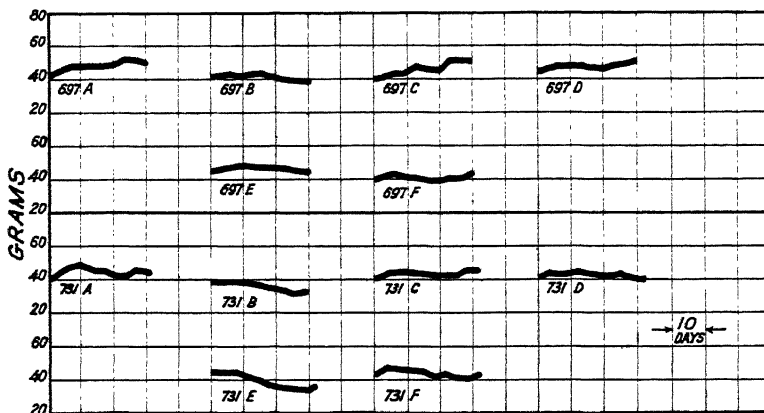


FIG. 7.—Growth curves of rats fed pork cracklings as the source of protein in their diet. Rats numbered 697 were fed a ration containing 15 per cent of protein from one lot of cracklings; rats numbered 731 received 20 per cent of protein from another lot of cracklings

loss. It may be recalled that 5 per cent of protein of good quality is adequate for maintenance and 10 per cent for approximately normal growth.

CALF LUNGS

A ration containing 10 per cent of protein from calf lungs was fed to rats numbered 725 (fig. 8 and Table 3). These rats made fair,

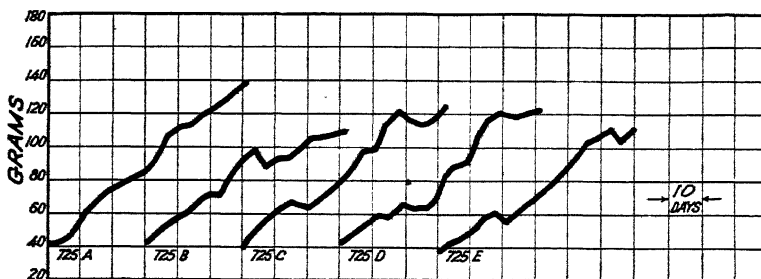


FIG. 8.—Growth curves of rats fed a ration containing 10 per cent of protein from calf lungs

though somewhat subnormal, growth. The growth curves are rather irregular, but the reason is not apparent. From Table 3 it appears that the rats getting 10 per cent of protein from calf lungs made an average gain in weight of 2.02 gm. for each gram of protein consumed during a period of 30 days as compared with a gain of 2.86 gm. for the rats getting 10 per cent of veal protein.

TABLE 3.—*Results of feeding tests with ox palates, hog snouts, cracklings, and calf lungs as sources of protein in the diet of rats*

Source of protein	Protein in ration	Rat No.	Duration of test	Initial weight	Final weight	Gain (+) or loss (-) in weight	Total feed consumed	Gain in weight per gram of protein consumed	
								36 days	60 days
	Percent		Days	Grams	Grams	Grams	Grams	Grams	Grams
Ox palates.....	10	678 A	60	40	55	+15	229	1.03	0.66
Do.....	10	678 B	60	39	58	+19	205	1.28	.93
Do.....	10	678 C	60	39	57	+18	248	1.17	.73
Do.....	10	678 D	62	40	61	+21	263	1.11	.82
Do.....	10	678 E	62	38	76	+38	296	1.50	1.18
Do.....	10	678 F	61	38	52	+14	208	1.20	.73
Average gains.....						+21		1.22	.84
Ox palates.....	15	679 A	61	39	81	+42	369	1.23	.90
Do.....	15	679 B	61	38	67	+29	253	1.01	.77
Do.....	15	679 C	61	39	72	+33	302	1.19	.72
Do.....	15	679 D	61	38	106	+68	361	1.32	1.26
Do.....	15	679 E	60	39	64	+25	244	.97	.68
Do.....	15	679 F	60	38	63	+25	265	1.10	.63
Average gains.....						+37		1.14	.83
Hog snouts.....	10	682 A	29	45	40	-5	118		
Do.....	10	682 B	29	43	56	+13	127		
Do.....	10	682 C	29	41	44	+3	115		
Do.....	10	682 D	29	43	45	+2	112		
Do.....	10	682 E	29	39	43	+4	107		
Do.....	10	682 F	29	42	47	+5	121		
Do.....	15	683 A	29	45	55	+10	124		
Do.....	15	683 B	29	45	46	+1	109		
Do.....	15	683 C	29	43	54	+11	123		
Do.....	15	683 D	29	41	50	+9	123		
Do.....	15	683 E	29	40	47	+7	106		
Do.....	15	683 F	29	45	49	+4	128		
Cracklings No. 1269.....	15	697 A	30	43	50	+7	100		
Do.....	15	697 B	30	42	39	-3	80		
Do.....	15	697 C	30	40	51	+11	100		
Do.....	15	697 D	30	45	51	+6	92		
Do.....	15	697 E	30	45	45		91		
Do.....	15	697 F	30	40	43	+3	90		
Cracklings No. 1284.....	20	731 A	31	39	44	+5	106		
Do.....	20	731 B	29	39	33	-6	96		
Do.....	20	731 C	32	40	46	+6	98		
Do.....	20	731 D	32	41	40	-1	129		
Do.....	20	731 E	33	45	37	-8	109		
Do.....	20	731 F	32	43	43		115		
Calf lungs.....	10	725 A	61	40	140	+100	549	2.10	1.84
Do.....	10	725 B	62	42	110	+78	445	2.23	1.55
Do.....	10	725 C	62	39	125	+86	461	2.06	1.83
Do.....	10	725 D	62	42	122	+80	444	1.75	1.83
Do.....	10	725 E	60	38	112	+74	381	1.97	1.94
Average gains.....						+84		2.02	1.80
Veal.....	10	665 A	60	41	214	+173	715	3.65	2.42
Do.....	10	665 B	60	44	188	+144	629	2.90	2.29
Do.....	10	665 C	60	40	165	+125	522	2.90	2.39
Do.....	10	665 E	60	44	185	+141	629	2.92	2.24
Do.....	10	665 F	60	41	163	+122	505	2.53	2.42
Average gains.....						+141		2.86	2.35

DISCUSSION OF RESULTS

In the experiments reported in this paper each meat product was the only source of protein in a ration which was adequate in other respects for growth, and the results obtained indicate the apparent value of a product as a source of protein for maintenance and growth only under these conditions. Although a protein may be inadequate

for growth when used alone in a ration, owing to a deficiency of one or more essential amino acids, the same protein may give much better results when fed in combination with other proteins which supply the lacking amino acids.

BEEF EXTRACT

A ration containing 18 per cent of so-called "crude protein" (nitrogen $\times 6.25$) from beef extract, but in reality only approximately 9 per cent of true protein compounds, was fed to young and to mature rats with very unsatisfactory results. In neither instance was this ration adequate even for maintenance, much less for growth, although only 5 per cent of protein of good quality is required for maintenance.

Although it appears that the nitrogenous compounds in beef extract are of poor nutritive quality, it is not to be inferred that the product has no value in the diet. Beef extract represents in high concentration the flavoring constituents of beef, and for that reason it has a distinct value for culinary purposes. Beef extract is also rich in phosphates, but its chief value appears to lie in the stimulating effect which it exerts upon the digestive processes.

BLOOD

The experiments with both dried blood and hemoglobin are unsatisfactory as a measure of the nutritive value of the protein in these products on account of the dislike which the rats showed for them. This aversion was indicated by the behavior of the rats but more particularly by the record of the food intake. Various expedients were used to increase food consumption, but without much success. It appears that the albino rat is not a satisfactory animal for the estimation of the nutritive value of dried blood and hemoglobin, and that the information must be obtained with other animals.

The experiments with dried serum show that 10 per cent of protein from that source was sufficient only for very slow growth in rats, but 15 per cent protein induced fairly satisfactory, though not normal growth. Since 10 per cent of muscle protein is practically sufficient for normal growth, it appears that serum protein has a much lower nutritive value.

OX PALATES

The experiments reported indicate that the protein in ox palates is deficient in certain essential amino acids, since rations containing 10 and 15 per cent⁴ respectively, of protein from this source induced only very slow growth. These results were to have been expected since ox palates consist chiefly of connective tissue with a small proportion of muscle. The fact that the protein in ox palates is fairly well digested (8) is, of course, not a correct indication of its nutritive value.

HOG SNOUTS

Rations containing 10 and 15 per cent, respectively, of protein from hog snouts were only slightly more than adequate for maintenance purposes. Since the hog snouts used consisted almost entirely of connective tissue and fat, and apparently no muscle tissue, these results were to have been expected.

⁴ Ten per cent of animal protein of good quality, such as that in muscle tissue, is practically adequate for normal growth in albino rats.

CRACKLINGS

Rations containing 15 and 20 per cent, respectively, of protein from two lots of cracklings proved to be only barely adequate for maintenance. Since these cracklings consisted chiefly of connective tissue and only a very little muscle tissue, the results are as expected. In view of these findings, statements that cracklings have as high nutritive value as tankage for feeding hogs seem open to question.

SUMMARY

In this paper are reported the results of feeding tests with rats to determine the relative nutritive values of the proteins in several meat products when each was the sole source of protein in the diet.

The total nitrogenous compounds in beef extract were found to have a very low value for maintenance and growth in rats. A ration containing 18 per cent of so-called "crude protein" (nitrogen \times 6.25), or approximately 9 per cent of true protein, was inadequate for maintenance.

Dried blood and hemoglobin were very distasteful to rats, and food consumption was so low that no statement concerning the nutritive value of the protein in these products is warranted.

Serum protein had a considerably lower nutritive value than muscle protein. Fifteen per cent of serum protein was somewhat inadequate for optimal growth.

The protein in ox palates was evidently deficient in certain amino acids, since 15 per cent of protein from this source induced only very slow growth.

Hog snouts and pork cracklings also contained incomplete proteins, since 15 per cent of protein from either source was adequate only for maintenance.

The protein in calf lungs was of fair quality, 10 per cent being sufficient for moderate growth.

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THE GROWTH OF *OPHIOBOLUS GRAMINIS* SACC. IN RELATION TO HYDROGEN-ION CONCENTRATION¹

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INTRODUCTION

Several investigators have observed an apparent influence of soil reaction on the occurrence and severity of the take-all disease of wheat caused by *Ophiobolus graminis* Sacc. In general, it has been considered that alkaline soils favor the disease, but this point has not been definitely settled. A study of the literature shows that the experimental data dealing with this problem are rather incomplete, and that the vague term "alkaline soil" generally refers to soil to which alkaline substances, particularly lime, have been added at one time or another. With the exception of one case, all observations have been made in the field and, as no hydrogen-ion determinations have been made for the soils in question, it is impossible to correlate the occurrence and severity of the disease with active acidity or alkalinity.

Recently, Kirby (7, 8)³ and Davis (4) have studied the influence of the reaction of nutritive media on the growth of the parasite in pure culture. Kirby concluded that the fungus grows best on a highly alkaline medium, whereas Davis's results indicate that a neutral or slightly acid medium is most favorable for growth. Their experiments were rather limited, and in view of this fact it is difficult to draw definite conclusions from their results. The work on this phase of the subject has been summarized by McKinney (9).

In order to gain additional information on this problem and to explain, if possible, some of the apparent inconsistencies, it seemed important first to conduct a series of experiments with the parasite in pure culture. Accordingly, the growth of the fungus has been studied under different hydrogen-ion concentrations on each of several synthetic and nonsynthetic types of liquid and solid media and under different environmental conditions. The results of these experiments are presented in this paper.

MATERIALS AND TECHNIQUE

ORGANISM

A single-spore strain of *Ophiobolus graminis* was used throughout this study. The fungus was isolated by R. S. Kirby from a single ascospore obtained from a wheat plant growing in the State of New York and affected with the take-all disease. A culture of this single-spore isolation was furnished to H. H. McKinney. He and R. J. Davis have kept the fungus in prime condition by repeated passage

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³ Reference is made by number (italic) to "Literature cited," p. 871.

through wheat plants and by single ascospore isolations from the fungus growing in pure culture.

Potato-dextrose agar proved to be the most favorable medium, and stock cultures were made in both test tubes and Petri dishes. The medium was made in the usual way: Potatoes 300 gm., dextrose 30 gm., agar 20 gm., water 1,000 c. c. All stock cultures were allowed to grow at room temperature either in the dark or in very subdued light, and only young and vigorous growing cultures were used as sources of inoculum. An incubation interval of 14 to 21 days for stock cultures proved satisfactory for all purposes.

METHOD OF CULTURE

The stock nutrient media for the experiments reported in this paper were made on the basis of a "2X" concentration; that is, 500 c. c. of water were used instead of 1,000 c. c. as specified in the formulae. This method, which has been described in detail by Karrer and Webb (6), permits dilutions of the various solutions by the addition of regulated quantities of acid or alkali and water for the adjustment of hydrogen-ion concentrations without disturbing the concentrations of the various constituents. The acid and alkali employed were HCl and NaOH. A strength of N/5 was very satisfactory always for the acid and, in most cases, for the alkali. In a few instances, however, when relatively large quantities of alkali were desired, a strength of N/1 was necessary.

Erlenmeyer culture flasks of 150 c. c. capacity were used, and in all cases each flask contained 50 c. c. of the nutrient solution. A 25 c. c. portion of the particular (2X) culture medium was pipetted into each flask, and sufficient water to equal the difference between 50 c. c. and the sum of the volumes of the nutrient medium and the acid or alkali was added. The flasks were plugged with cotton and autoclaved for 30 minutes at 15 pounds pressure. Similarly, the acid and the alkali were sterilized separately. After cooling, the cultures were removed to a culture chamber and definite quantities of sterile acid and alkali were added with sterile, graduated pipettes. The separate sterilization of the acid, the alkali, and the nutrient medium minimizes any reaction on the part of the sugar. Moreover, the final volume of the culture solutions in each case represents a dilution of the constituents comparable with that specified in the original formula. The solutions were allowed to stand at least for a period of 24 hours in order to reach a state of equilibrium before the hydrogen-ion determinations were made. Certain color transformations occurred from the addition of alkali, but these will be discussed later.

Similar methods were adopted for the solid media. Large quantities of the 2X culture fluid to which 2 per cent bacto agar had been added were pipetted, while warm, into 1,000 c. c. flasks. Calculated quantities of water were added to each, the flasks were plugged, and the cultures were sterilized intermittently on three successive days for a period of 15 minutes at 13 pounds pressure. After the third sterilization and before cooling, regulated quantities of acid or alkali were added to the flasks and the solutions were vigorously shaken. These stock solutions then were poured into sterile Petri dishes, and usually there were 6 to 8 plates for each reaction. The plates employed were 9 cm. in diameter and the volume of medium in each

ranged from 20 to 25 c. c. Hydrogen-ion determinations were made immediately with the remaining portions of the stock solutions. Transformations in color and difficulties with setting were encountered in certain instances, but these will be discussed later. Usually 6 to 8 liters of the 2X solution or of the 2X agar were made at the same time, so all the data represent uniformity for a particular medium.

All operations pertaining to the culture work were performed in a culture chamber, and every precaution was taken to prevent contaminations. The interior parts of the culture chamber were washed with 1:1,000 HgCl₂ and the cultures and all necessary apparatus were then deposited. Dust particles within the chamber were precipitated by means of live steam flowing into the chamber for a period of several minutes. During long operations, live steam was permitted to flow periodically and no cultures were disturbed until everything had become settled. It is of interest to note that only about 2 per cent of the flask cultures became contaminated. Petri-dish cultures showed a somewhat higher percentage of contamination on account of the fact that the covers were removed daily to permit accurate measurements of the colonies.

CULTURE MEDIA

The adjusted P_H values for each of the liquid and solid media used are given in Table 1, together with the quantity of acid or alkali necessary for such adjustment.

The formula for the potato-dextrose decoction is as follows: 300 gm. of potatoes, 30 gm. of dextrose, and 1,000 c. c. of water. The potatoes were peeled, cut into small pieces, autoclaved for one hour at 15 pounds pressure, and filtered through absorbent cotton while hot. Dextrose was added, the solution was thoroughly stirred, and regulated portions were pipetted into flasks. The decoction always tested P_H 5.7 or P_H 5.8, and the buffer action was very good. With a dilution of 10 times, the decoction tested P_H 6.0, and with a dilution of 30 times, it tested P_H 6.2.

Czapek's full nutrient solution was made according to the formula used by Karrer and Webb (6): MgSO₄, 0.5 gm.; KH₂PO₄, 1.0 gm.; KCl, 0.5 gm.; NaNO₃, 2.0 gm.; FeSO₄, 0.01 gm.; cane sugar 30 gm.; H₂O, 1,000 c. c. This nutrient solution also was used in two modified forms. In one case nucleic acid was substituted for the sodium nitrate. In the other case, caramelization was produced by prolonged autoclaving as follows: A large quantity of the nutrient solution was made upon a basis of 10X concentration, autoclaved for two or four hours at 15 pounds pressure, diluted to 2X, and pipetted into the culture flasks.

The corn-meal decoction was prepared in the following manner: 150 gm. of corn meal were added to 1,000 c. c. of water. The mixture was then heated over a flame, brought to a slow boil, filtered twice through two thicknesses of cheesecloth, and finally filtered through absorbent cotton.

Solid media were made in all cases by the single addition of bacto agar to the liquid media at the rate of 20 gm. per liter.

A knowledge of the factors or conditions which stimulate growth in the alkaline cultures of potato-dextrose agar should prove valuable. Reference is made to the increasing browning with increasing additions

of alkali. This in itself indicated that chemical changes were taking place, but little definite information relative to the occurrence and the degree of such changes in culture media is available.

TABLE 1.—*Liquid and solid media adjusted to various hydrogen-ion concentrations by regulated additions of N/5 hydrochloric acid or N/5 sodium hydroxide*

2N solution	HCl or NaOH N/5	H ₂ O	Total	Hydrogen-ion concentration, P _H								
				Potato dextrose		Corn meal		Czapek's solution	Czapek's nucleic acid		Czapek's caramelized	
				Decoc-tion	2 per cent agar	Decoc-tion	2 per cent agar		Solu-tion	2 per cent agar	Solu-tion	2 per cent agar
HCl												
C. c.	C. c.	C. c.	C. c.									
25	5.00	20.00	50	2.50	3.20					2.3		
25	4.00	21.00	50	2.95	3.40							2.3
25	3.50	21.50	50					2.0		2.5		
25	3.00	22.00	50	3.50	3.70							
25	2.50	22.50	50									2.6
25	2.00	23.00	50	4.00	4.10					3.0		
25	1.20	23.80	50									3.1
25	1.00	24.00	50					2.6	2.6	3.6	2.5	
25	.75	24.25	50	4.55	4.60	2.70						
25	.60	24.40	50			3.20						3.4
25	.50	24.50	50									
25	.40	24.60	50			3.50		3.0	3.6	4.7		
25	.30	24.70	50			3.60	4.0				3.0	3.7
25	.20	24.80	50			3.90	4.4	3.5	4.2	5.1		
25	.10	24.90	50			4.20	5.2				3.3	3.8
25	.00	25.00	50	5.80	5.30	5.30	5.8	4.6	5.5	5.5	3.3	3.9
NaOH												
25	.05	24.95	50			6.60	6.8					
25	.10	24.90	50			6.80	7.1	5.5	5.7	5.7	3.4	4.3
25	.20	24.80	50			7.80	7.5				3.6	
25	.30	24.70	50	6.50	5.60	8.20	8.1					5.1
25	.40	24.60	50								4.7	
25	.50	24.50	50	6.95	5.80	8.50	8.3					
25	.60	24.40	50									5.7
25	.75	24.25	50	7.40	6.70	9.00	8.4	6.6	6.6	6.5	5.5	
25	1.00	24.00	50								5.8	6.3
25	1.10	23.90	50	7.70				7.0				
25	1.30	23.70	50						7.0	6.7		
25	1.50	23.50	50	8.30							6.5	6.6
25	1.60	23.40	50					7.6				
25	1.75	23.25	50		7.00				7.9	6.9		
25	1.90	23.10	50					8.0				
25	2.00	23.00	50	8.40	8.00	10.30	9.0		8.4	7.4	6.8	6.9
25	2.50	22.50	50	8.80				8.7				
25	3.00	22.00	50						9.5	8.5	7.3	7.8
25	4.00	21.00	50			11.20	9.3	9.8+			9.2	8.8
25	5.00	20.00	50	9.50	9.10			9.8+	10.8	9.5		
25	6.00	19.00	50			11.80	9.8	9.8+			9.6	9.5
25	7.00	18.00	50						11.4	10.1		
25	8.00	17.00	50	9.6+	9.50	12.10	10.1				10.2	
25	10.00	15.00	50	9.6++	9.60		10.4		11.7	10.5	10.7	9.6
25	15.00	10.00	50				10.8			11.3	11.0	9.7
25	16.65	8.35	50		9.60							
25	20.00	5.00	50				11.7					
25	20.80	4.20	50		9.60							
25	25.00	0.00	50		10.30					11.8		10.9

HYDROGEN-ION DETERMINATIONS

Hydrogen-ion determinations of all liquid media were made at room temperature and of all solid media at 60° to 70° C. by both the colorimetric and electrometric methods, as described in detail by Clark (9). The liquid media were allowed to stand 24 hours at room temperature before being tested. The various types of solid media were held in a water bath at 60° to 70° C., following the last steril-

ization and subsequent additions of either acid or alkali, and were tested as soon as possible. Such factors as time and temperature proved extremely important. The presence of color necessitated the use of a comparator and colorimeter, the intensity of the color determining which to use. A standard instrument, modified and calibrated to meet the requirements of this work in accordance with the method described by Duggar (5), was employed. Czapek's solution gave a bluish-green color upon the addition of the bromophenol blue indicator, and it was impossible to get good agreements between these colors and those of the buffer standard solutions, particularly near P_H 3.0. Electrometric determinations were made for the cultures beyond the alkaline range of the indicators, and sufficient electrometric tests also were made on the culture solutions and on the standard buffer solutions to verify the colorimetric method.

The methods employed for the final hydrogen-ion values of the substrate were similar to those for the initial determinations. The fungus generally produced a definite mat in the favorable cultures of the liquid media, and filtration therefore was simple. The filtrates were tested both colorimetrically and electrometrically. In the case of the solid media, the substrates were cut into small pieces and the agar blocks from duplicate cultures were transferred to clean Erlenmeyer flasks. The flasks were placed in a water bath with a temperature of 60° to 70° C. and kept there until the substrates were liquefied. The fragments of fungous mats floated to the surface and were removed.

Marked changes in the reaction of alkaline solid media occurred at the temperatures employed for liquefaction of the agar. The changes varied more or less directly with the amount of alkali present, the temperature employed, and the time involved. When agar cultures were kept in a water bath at 60 to 70° C. while the hydrogen-ion determinations were being made, it soon became apparent that the electrometric and colorimetric methods of determination gave different results. A period of 15 to 20 minutes generally was necessary for equilibrium to be reached by the electrometric method, and the values so obtained were usually a few tenths of a P_H less than those obtained immediately by the colorimetric method. If, however, colorimetric determinations were made at the time that the electrometric readings were made, the values of the two methods were practically identical. More uniform results were obtained when the cultures were kept in a water bath at a constant temperature of 60° C. than when they were subjected to live steam in an Arnold sterilizer for short periods. Intense browning occurred in the most alkaline cultures, and rather high dilutions were necessary for the colorimetric determinations. The immediate colorimetric figures are believed to represent more nearly the exact reactions of the poured plates than do the delayed electrometric values. The speed with which colorimetric determinations can be made therefore reduces the time factor to a minimum, and this is a decided point in favor of this method for agar determinations. It is regrettable that the range of indicators is limited on the alkaline side and that wider application of this method is thus prevented at the present time.

The hydrogen-ion values in the tables represent initial and final determinations for both inoculations and controls. Reactions extending almost throughout the entire range were obtained with each

medium, and especial attention has been given to the study of the influence of slight acidity, of neutrality, and of slight alkalinity on the growth of the organism. Aside from these desired concentrations, the range has been conveniently but not equally subdivided.

INOCULATION AND INCUBATION

The inoculum consisted of young mycelium grown on potato-dextrose agar in Petri dishes. The age of the cultures generally varied from 14 to 21 days, and the mycelium was always taken from a concentric zone about 2 cm. in width near the periphery of the fungus colony. Agar containing such mycelium was cut into small blocks about 4 mm. square, and one such inoculum block was placed in each culture. With the liquid media, some of the blocks would naturally sink and others would not. However, by rotating the flasks vigorously, all of the blocks were made to sink. The growth data for the liquid media, therefore, represent growth which initially was submerged.

Similarly, an inoculum block was placed on the surface of the agar in the center of each Petri dish. Adequate uninoculated controls were included for the experiments involving both liquid and solid media.

The cultures for the most part were incubated in large glass chambers located in a greenhouse, and the temperature and humidity in these chambers were controlled by automatic regulators. The cultures were placed in single rows on shelves near one of the glass walls of the chamber and were protected from direct sunlight by shades and screens. Certain of the cultures were incubated in the dark. Heavy black paper was properly cut, fitted around each flask, and held in place by a rubber band at the neck of the flask. Black hoods made of the same material were placed over these flasks and all light was thus excluded. The hoods were removed each day for a few minutes in order to promote aeration more or less comparable with that in the unhooded cultures. The Petri-dish cultures were placed in sterile paper bags and incubated in the laboratory incubators; the bags were removed under sterile culture-chamber conditions for a few minutes each day while growth data for the organism were being recorded.

During the late spring, temperatures were not easily controlled in the greenhouse chambers, and it became necessary to employ exclusively the incubators located in the laboratories. The temperatures in these incubators were regulated within 1 degree, but there was no control for humidity, and the cultures grew in total darkness.

CHEMICALS

High-grade chemicals were used throughout this investigation, and the most careful technic was employed in all instances. To eliminate the high acidity of the monobasic potassium phosphate, this salt was recrystallized until the Sørensen coefficient of P_H 4.529 for 1/15 molecular solution was obtained. Doubly distilled lake water, testing P_H 6.3 to P_H 6.5 distilled first from an ordinary still and finally from a Bourdillon still with a block-tin condenser, was used in all of the experiments reported. A few crystals of potassium bisulphate were used in the Bourdillon still, as modified and described by Bennett and Dickson (1).

GLASSWARE

The highest-grade flasks, pipettes, and burettes were used throughout the investigation. All glassware was thoroughly seasoned in the beginning by being autoclaved in chromic-acid cleaning mixture for a period of one hour. The glassware was scrubbed with soap and hot water, soaked in chromic-acid cleaning mixture, washed repeatedly in tap water, rinsed in singly and in doubly distilled water, and protected from dust until needed.

EXAMINATION OF CULTURES

The experiments involving liquid media were conducted either in duplicate or in triplicate, and those involving solid media generally included 6 to 8 plates at each reaction. All cultures were inspected daily and records made accordingly. The growth data presented in this paper represent averages of these cultures. Dry weight of mycelium has been used as the criterion of growth in liquid media and diameter of colony for that in solid media. The substrate and fungous mat together were removed from each culture and filtered by suction through a Büchner funnel on a previously dried and weighed filter paper. The fungous material was washed several times with distilled water, the papers were then folded so as to prevent loss of material, and dried in an oven at 100° C. until a constant weight was obtained. An interval of from 24 to 48 hours was adequate. A definite time interval was allowed for the weighing of each empty filter paper and each filter paper containing fungous material. Where growth was very poor, as in Czapek's solution, much more consistent results were obtained for growth than when the papers were weighed without consideration of this point.

EXPERIMENTAL RESULTS

In presenting the results of the study, the data on the growth on solid media are given first, followed by similar data for the corresponding liquid media. The length of the incubation period and the temperature proved to be important factors with a direct bearing on the results obtained. The effects of these factors are considered subsequently under respective subheadings.

GROWTH ON SOLID MEDIA ADJUSTED TO DIFFERENT REACTIONS

In general, *Ophiobolus graminis* grew better on a solid substrate than in a liquid one. The rates of growth and the total quantities of growth within the favorable P_H ranges were more uniform for the several solid media tested than they were for the corresponding liquid media. Different criteria for growth necessarily were employed; that is, diameter of colony for the solid media and dry weight of the fungus for the liquid media, but the two methods were found to give more or less comparable results. The growth curves for the several solid media and the relations between the growth curves for the corresponding solid and liquid media will be discussed briefly.

The physical nature of the medium influenced the range of growth and the magnitude of growth quantities more in the case of potato dextrose than in any other. The range of growth was very restricted in potato-dextrose decoction, extending only from P_H 3.6 to P_H 7.7.

On potato-dextrose agar, however, the range expanded slightly on the acid side and markedly on the alkaline side, the growth range extending from P_H 3.2 to P_H 10.3. Two series of cultures on potato-dextrose agar were incubated in the dark at $24^\circ C$. The cultures were examined daily and remarkably consistent data were obtained, as shown by Table 2. A period of two days was necessary for growth to become measurable, and the growth increased and the limits expanded uniformly with increase of incubation period. At the end of seven days, growth occurred in all cultures between P_H 3.2 and P_H 9.6, and the colonies entirely filled the plates of cultures testing P_H 5.6 and P_H 5.9. Growth was very small in the most acid cultures, increased rather gradually to a maximum at P_H 5.6 to P_H 5.9, decreased to a minimum at P_H 9.2, and finally exhibited a tendency to increase in the most alkaline cultures. The growth curve for the second series is presented in Figure 1 with those from other solid media. The bimodal tendency was very pronounced and consistent in both series. An additional 6-day period of

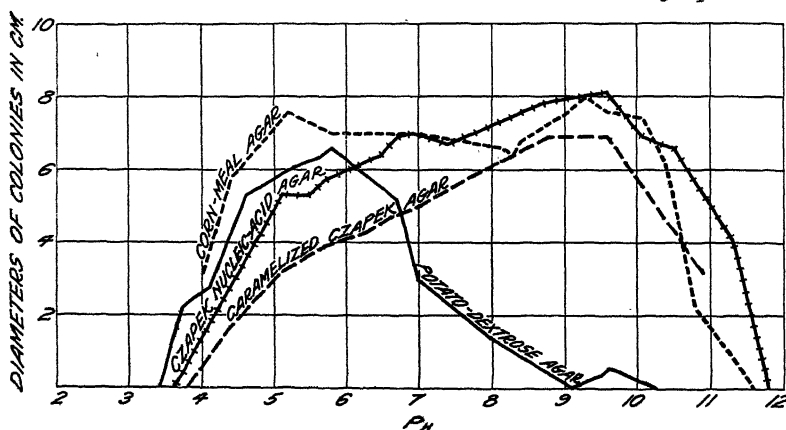


FIG. 1.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* on four solid media in a period of six days in the dark at $24^\circ C$.

incubation furnished very similar relations. The range of growth remained the same as before, i. e., P_H 3.2 to P_H 9.6; the bimodal tendency became more conspicuous, and growth covered the entire plates of those cultures testing P_H 4.8 to P_H 7.1 and P_H 9.6.

Caramelization of the potato-dextrose agar occurred in the most alkaline cultures and increased somewhat proportionally, as determined by the intensity of the brown coloration, with the quantity of alkali added. The stimulation of fungous growth seemed to be correlated more or less directly with this phenomenon up to a certain point. Cultures testing P_H 9.1 and P_H 9.2 were the most alkaline of these which did not show caramelization, and here the growth curve reached a minimum. Between the reactions of P_H 9.2 and P_H 10.3, the growth curve rose consistently and reached a maximum near P_H 9.5. Slight caramelization of the alkaline cultures in Czapek's solution likewise caused marked stimulation of fungous growth, whereas pronounced caramelization proved toxic to the fungus. Undoubtedly the conditions for growth in caramelized cultures are considerably different from those in noncaramelized cultures.

TABLE 2.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, on potato-dextrose agar for various periods in the dark at 24° C.*

Hydrogen-ion concentration, P _H				Average diameter of colonies in centimeters after—															
After 7 days				Final															
Initial	Controls	Inocu- lated	Controls	Inocu- lated	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days
3.0	3.1	3.1	3.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.2	3.2	3.2	3.2	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.3	3.3	3.3	3.3	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.4	3.4	3.4	3.4	3.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.5	3.5	3.5	3.5	3.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.6	3.6	3.6	3.6	3.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.7	3.7	3.7	3.7	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.8	3.8	3.8	3.8	3.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.9	3.9	3.9	3.9	3.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.0	4.0	4.0	4.0	4.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.1	4.1	4.1	4.1	4.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.2	4.2	4.2	4.2	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.3	4.3	4.3	4.3	4.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.4	4.4	4.4	4.4	4.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.5	4.5	4.5	4.5	4.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.6	4.6	4.6	4.6	4.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.7	4.7	4.7	4.7	4.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.8	4.8	4.8	4.8	4.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.9	4.9	4.9	4.9	4.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.0	5.0	5.0	5.0	5.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.1	5.1	5.1	5.1	5.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.2	5.2	5.2	5.2	5.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.3	5.3	5.3	5.3	5.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.4	5.4	5.4	5.4	5.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.5	5.5	5.5	5.5	5.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.6	5.6	5.6	5.6	5.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.7	5.7	5.7	5.7	5.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.8	5.8	5.8	5.8	5.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.9	5.9	5.9	5.9	5.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.0	6.0	6.0	6.0	6.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.1	6.1	6.1	6.1	6.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.2	6.2	6.2	6.2	6.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.3	6.3	6.3	6.3	6.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.4	6.4	6.4	6.4	6.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.5	6.5	6.5	6.5	6.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.6	6.6	6.6	6.6	6.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.7	6.7	6.7	6.7	6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.8	6.8	6.8	6.8	6.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.9	6.9	6.9	6.9	6.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.0	7.0	7.0	7.0	7.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.1	7.1	7.1	7.1	7.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.2	7.2	7.2	7.2	7.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.3	7.3	7.3	7.3	7.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.4	7.4	7.4	7.4	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.5	7.5	7.5	7.5	7.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.6	7.6	7.6	7.6	7.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.7	7.7	7.7	7.7	7.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.8	7.8	7.8	7.8	7.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.9	7.9	7.9	7.9	7.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.0	8.0	8.0	8.0	8.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.1	8.1	8.1	8.1	8.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.2	8.2	8.2	8.2	8.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.3	8.3	8.3	8.3	8.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.4	8.4	8.4	8.4	8.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.5	8.5	8.5	8.5	8.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.6	8.6	8.6	8.6	8.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.7	8.7	8.7	8.7	8.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.8	8.8	8.8	8.8	8.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.9	8.9	8.9	8.9	8.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.0	9.0	9.0	9.0	9.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.1	9.1	9.1	9.1	9.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.2	9.2	9.2	9.2	9.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.3	9.3	9.3	9.3	9.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.4	9.4	9.4	9.4	9.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FIRST SERIES										SECOND SERIES									
Average diameter of colonies in centimeters after—										Average diameter of colonies in centimeters after—									
Final										Final									
Initial	Controls	Inocu- lated	Controls	Inocu- lated	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days
3.2	3.2	3.2	3.1	3.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.4	3.4	3.4	3.4	3.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.7	3.7	3.7	3.7	3.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.1	4.1	4.1	4.1	4.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.6	4.6	4.6	4.6	4.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.3	5.3	5.3	5.3	5.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.6	5.6	5.6	5.6	5.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.8	5.8	5.8	5.8	5.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.7	6.7	6.7	6.7	6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.0	7.0	7.0	7.0	7.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.0	8.0	8.0	8.0	8.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.1	9.1	9.1	9.1	9.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.5	9.5	9.5	9.5	9.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.6	9.6	9.6	9.6	9.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.6	9.6	9.6	9.6	9.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.6	9.6	9.6	9.6	9.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10.3	10.3	10.3	10.3	10.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* F=traces of growth on edges of inoculum block.

* E=growth entirely covered 9-centimeter Petri dish.

Growth was very rapid on corn-meal agar at 24° C., and the fungus colonies covered the entire plates at the majority of the different initial reactions within a period of eight days. The results given in Table 3, and represented by one of the curves in Figure 1, show that the growth range extended to the limits of the experiment, i. e., P_H 4.0 to P_H 11.7, and that maxima occurred at P_H 5.2 and at P_H 9.3. A diminution in growth appeared at P_H 8.3 and this value for the corn-meal agar is very close to that furnished by the corn-meal decoction. This point may prove to be the isoelectric point of this medium, as determined by the growth of the fungus, but no definite statement can be made at this time. With six additional days of incubation, the range of best growth expanded and the colonies covered the entire surfaces of the cultures, ranging from P_H 4 to P_H 10.4. The most alkaline culture, possessing a reaction of P_H 11.7, was not far removed from the point of inhibition of growth. The most acid culture, i. e., P_H 4.0, did not even approach such a value.

The modified Czapek's full nutrient agar containing nucleic acid in place of $NaNO_3$ furnished interesting growth data, as shown by Table 4 and one curve in Figure 1. Growth on the solid medium was very good and much better than that in the corresponding liquid medium. The rate and the quantity of growth in the favorable cultures resembled that produced by potato-dextrose agar. Incubated at 24° C. in the dark for a period of seven days' growth occurred in all the cultures ranging from P_H 4.7 to P_H 11.3, and the colonies entirely covered the plates within the range of P_H 6.9 to P_H 10.1. Maximum growth, however, was obtained at P_H 9.6 on the sixth day of incubation, which was the day before the colonies covered the plates within the range mentioned. With prolonged intervals of incubation, the range of best growth expanded until it extended from P_H 4.7 to P_H 11.3 on the thirteenth day. No growth was obtained at P_H 3.0 or at P_H 11.8 and only slight growth at P_H 3.6.

Growth data from cultures on caramelized Czapek's agar (Table 4) resembled very closely those from cultures on Czapek's nucleic-acid agar. There is a striking similarity between the results of the two series, as shown by the growth curves of Figure 1. Because of the high acidity or of other chemical changes produced during the process of caramelization, the caramelized Czapek agar did not gel to the usual degree. Measurements of growth were particularly difficult to make under such conditions, especially as the fungus tended to grow less superficially and as the color contrast between fungous growth and medium was very slight. The range of growth extended from P_H 3.8 to the limit of the experiment, namely, P_H 10.9, and maximum growth occurred from P_H 8.8 to P_H 9.6+. The curve ascended to and descended from this conspicuous peak rather rapidly. The most acid cultures were incubated for an additional 5-day period, but the increase of growth in these cultures was remarkably small. These results may be found by reference to Table 4.

A considerable number of shifts in the reaction of the solid substrates were induced by the fungus during growth. In potato-dextrose agar, Table 2, the shifts were generally in the direction of alkalinity and the degree of the shift varied somewhat with the initial reaction. The changes were consistent for both series of potato-dextrose agar after 7 days' growth, but reversible shifts

TABLE 3.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, on corn-meal agar of different initial P_H values for various periods in the dark at 24° C.*

Hydrogen-ion concentration, P _H				Average diameter of colonies in centimeters after—													
Initial P _H	Final (7 days)		Final (14 days)		2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
	Controls	Inoculated	Controls	Inoculated													
4.0	4.1	4.5	4.1	4.9	0.3	1.3	2.0	2.5	3.1	3.9	4.7	5.4	6.0	6.7	7.7	8.1	8.6
4.4	4.4	5.6	4.5	5.5	1.7	2.6	3.6	4.6	5.8	6.9	8.6						
5.2	5.3	7.0	5.2	6.8	1.7	3.1	4.6	6.0	7.5	8.4	8.6						
5.8	5.8	7.3	5.5	7.2	2.1	3.1	4.5	5.8	7.0	8.4	8.4						
6.8	6.8	7.6	6.9	7.4	2.0	3.0	4.4	5.6	6.9	8.3	8.3						
7.1	7.2	7.7	7.2	7.5	1.9	3.0	4.3	5.4	6.8	8.0	8.0						
7.5	7.5	7.9	7.9	7.9	1.7	3.0	4.2	5.4	6.8	8.0	8.0						
8.1	8.0	8.1	8.2	8.2	1.8	2.9	4.2	5.5	6.6	8.0	8.0						
8.3	8.3	8.3	8.4	8.4	1.6	2.8	4.0	5.2	6.4	7.9	7.9						
8.4	8.5	8.4	8.6	8.4	1.8	2.9	4.2	5.5	6.8	8.3	8.3						
9.0	8.9	8.7	8.9	8.7	1.7	3.0	4.7	6.0	7.5	8.7	8.7						
9.3	9.0	8.8	9.2	8.9	1.7	3.4	4.8	6.3	8.0	8.7	8.7						
9.6	9.1	8.9	9.3	9.2	1.8	2.9	4.4	5.9	7.6	8.7	8.7						
10.1	9.2	9.1	9.4	9.3	1.7	3.0	4.4	5.9	7.4	8.7	8.7						
10.4	9.3	9.3	9.5	9.4	1.4	2.5	3.7	5.0	6.2	7.2	7.2						
10.8	9.5	9.5	9.6	9.5	^b F	^b F	^b F	1.7	2.2	2.5	3.1	3.6	3.9	4.7	4.8	4.9	5.3
11.7	9.6	9.6	9.6+	9.6	0	0	^b F	^b F	^b F	^b F	3.5	3.7	3.9	4.9	1.0	1.2	1.2

• F = growth entirely covered 9.0 centimeter Petri dish.

• F = trace of growth on edges of inoculum block.

TABLE 4.—The growth of *Ophiobolus graminis*, and the changes in reaction induced by growth, on Czapek's modified agar of different initial P_H values for various periods in the dark at 24° C.

CZAPEK'S NUCLEIC-ACID AGAR

Hydrogen-ion concentration, P_H				Average diameter of colonies in centimeters after--													
Initial	After 7 days		After 17 days														
	Control	Inoculated		Control	Inoculated	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days
2.3					0	0	0	0	0	0	0	0	0	0	0	0	0
2.5					0	0	0	0	0	0	0	0	0	0	0	0	0
3.0					0	0	0	0	0	0	0	0	0	0	0	0	0
3.6		3.1		3.1	0	0	0	0	0	0	0	0	0	0	0	0	0
4.7		3.6		3.6	0	0	0	0	0	0	0	0	0	0	0	0	0
5.1		4.7		4.7	0	0	0	0	0	0	0	0	0	0	0	0	0
5.5		5.2		5.2	0	0	0	0	0	0	0	0	0	0	0	0	0
5.7		5.5		5.5	0	0	0	0	0	0	0	0	0	0	0	0	0
5.7		5.6		5.6	0	0	0	0	0	0	0	0	0	0	0	0	0
6.5		5.7		5.7	0	0	0	0	0	0	0	0	0	0	0	0	0
6.5		6.5		6.5	0	0	0	0	0	0	0	0	0	0	0	0	0
6.7		6.7		6.7	0	0	0	0	0	0	0	0	0	0	0	0	0
6.9		6.9		6.9	0	0	0	0	0	0	0	0	0	0	0	0	0
7.4		7.2		7.2	0	0	0	0	0	0	0	0	0	0	0	0	0
8.7		8.3		8.3	0	0	0	0	0	0	0	0	0	0	0	0	0
9.6		8.7		8.7	0	0	0	0	0	0	0	0	0	0	0	0	0
10.1		8.8		8.8	0	0	0	0	0	0	0	0	0	0	0	0	0
10.3		8.9		8.9	0	0	0	0	0	0	0	0	0	0	0	0	0
11.3		9.2		9.2	0	0	0	0	0	0	0	0	0	0	0	0	0
11.8		9.4		9.4	0	0	0	0	0	0	0	0	0	0	0	0	0

CZAPEK'S CARAMELIZED AGAR

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* F = trace of growth on edges of inoculum block.

* Cultures contaminated.

* E = growth entirely covered 9-centimeter dish.

appeared after 14 days' growth in the acid cultures of the first series which exhibited best growth. Growth was generally less in the second series than in the first series. The cultures either maintained their reactions or became more alkaline with prolonged incubation, and there were no indications of reversible shifts. The degree of the secondary shift in potato-dextrose agar was smaller as compared to that in potato-dextrose decoction. Buffer action is much stronger in potato-dextrose agar than in potato-dextrose decoction, and this may assist in explaining the variations.

Corn-meal agar, as influenced by growth of the fungus, showed changes in reaction of the medium somewhat similar to those of potato-dextrose agar. The reaction of the acid cultures shifted towards alkalinity and attained an alkaline value in many cases, whereas that of the alkaline ones either remained constant or became slightly less alkaline. Final determinations of the most alkaline uninoculated controls showed a considerable diminution of alkalinity upon standing seven days. These results are included in Table 3.

The fungus produced very slight changes or none at all in the Czapek agar containing nucleic acid and in the caramelized Czapek agar. Marked changes occurred in the reaction of the most alkaline inoculated cultures, but the changes were no greater than those in the corresponding uninoculated controls. Table 4 contains the data for the caramelized and the nucleic-acid Czapek agar.

TABLE 5.—The growth of *Ophiobolus graminis*, and the changes in reaction induced by growth, in potato-dextrose decoction of different initial P_H values, temperatures, and periods of incubation in the dark

Incubation		Hydrogen-ion concentration, P _H			Dry weight of fungous mat
Temper- ature, ...°C.	Time in days	Initial	Final		
			Control	Inocu- lated	
10	27	2.9	2.9	2.9	Gm.
		3.9	3.8	4.0	0.0000
		4.9	5.0	7.0	.0664
		5.8	5.8	7.4	.1456
		6.7	6.7	7.3	.1219
		7.1	6.9	7.4	.1382
		7.5	7.4	7.8	.1281
		8.4	8.2	7.9	.1002
		8.8	8.5	8.5	.0108
		2.9	2.9	2.9	.0000
		3.9	4.0	4.4	.0000
		4.9	4.9	7.2	.1378
		5.7	5.6	7.3	.2020
		6.5	6.4	7.2	.1971
24	12	6.9	6.8	7.3	.1880
		7.4	7.2	7.3	.1758
		8.3	8.1	8.1	.2123
					.0000

GROWTH ON LIQUID MEDIA ADJUSTED TO DIFFERENT REACTIONS

The results indicate that the influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* varies considerably, and depends to a large extent upon the nutritive relations and other factors. The organism is capable of growing throughout a wide range of hydrogen-ion concentrations in all media employed, except potato-dextrose decoction. Referring to Tables 5 and 6 and to

Figures 2, 3, and 4 it will be seen that on potato-dextrose decoction the fungus had a relatively restricted range, i. e., P_H 3.6 to P_H 7.7. Despite the fact, however, that such a narrow range for growth occurred in this medium, it consistently produced excellent growth at the favorable reactions, and therefore is regarded as the best medium employed in this study.

TABLE 6.—The growth of *Ophibolus graminis*, and the changes in reaction induced by growth, in potato-dextrose decoction in diffused light at different temperatures, periods of incubation, and initial P_H values

Incubation		Hydrogen-ion concentration, P _H			Dry weight of fungus mat
Temperature, °C.	Time in days	Initial	Final		
			Control	Inoculated	
10	12	2.9	2.8	2.8	Gm. 0.0000
		3.9	3.8	4.0	.0077
		4.9	4.9	5.1	.0100
		5.8	5.8	6.1	.0114
		6.7	6.6	6.9	.0189
		7.1	6.9	7.1	.0078
		7.5	7.4	7.1	.0070
		8.4	8.2	8.0	.0000
		2.9	3.0	2.9	.0000
		3.6	3.5	3.6	.0007
		4.1	4.1	4.4	.0243
		4.9	5.0	5.7	.0357
16	12	5.8	5.9	6.6	.0416
		6.4	6.5	6.8	.0529
		6.8	6.7	7.0	.0565
		7.2	7.0	7.1	.0617
		7.7	7.4	7.4	.0091
		8.4	8.2	8.0	.0000
		2.9	2.9	2.9	.0000
		3.6	3.5	3.6	.0065
		4.1	4.1	5.0	.0811
		4.9	4.9	6.6	.0717
		5.8	6.1	7.2	.0841
		6.4	6.2	7.3	.1226
20	12	6.8	6.6	7.6	.1405
		7.2	7.0	7.6	.1377
		7.7	7.3	7.3	.0107
		8.4	8.1	8.0	.0000
		2.9	3.0	3.0	.0000
		3.6	3.5	3.5	.0027
		4.1	4.1	4.4	.0326
		4.9	4.8	5.3	.0367
		5.8	5.7	6.3	.0471
		6.4	6.4	6.7	.0577
		6.8	6.8	6.9	.0584
		7.2	7.1	7.2	.0723
24	8	7.7	7.4	7.4	.0035
		8.4	8.3	8.2	.0000
		2.9	2.9	2.9	.0000
		3.9	4.1	4.7	.1053
		4.9	5.0	7.3	.1959
		5.7	6.1	7.4	.2021
		6.5	6.8	7.5	.1985
		6.9	6.8	7.5	.1884
		7.4	7.4	7.6	.1914
		8.3	8.1	8.1	.0000
		2.9	2.9	2.9	.0000
		3.6	3.6	3.6	.0000
28	12	4.1	4.1	3.9	.0114
		4.9	5.0	5.2	.0148
		5.8	5.8	5.9	.0344
		6.8	6.4	6.8	.0515
		7.2	6.8	6.8	.0228
		7.7	7.3	7.4	.0116
		8.4	7.9	-----	.0000

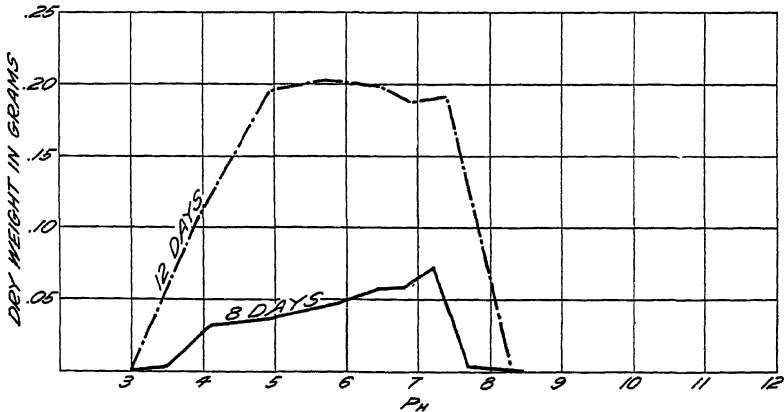


FIG. 2.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* in potato-dextrose decoction for 8 and 12 days in diffused light at 24° C.

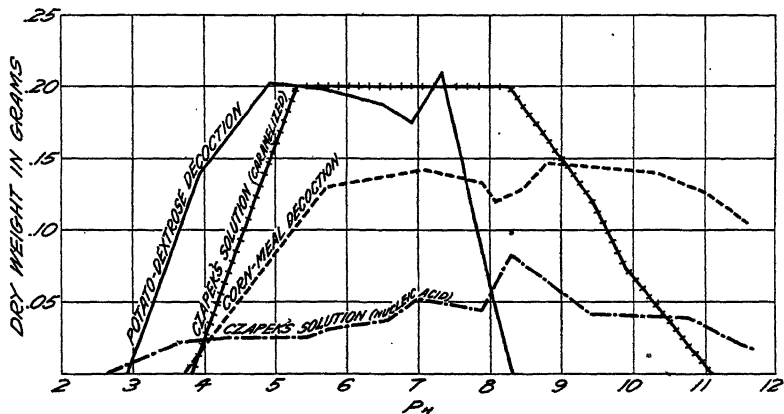


FIG. 3.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* in four liquid media in a period of 12 days in the dark at 24° C.

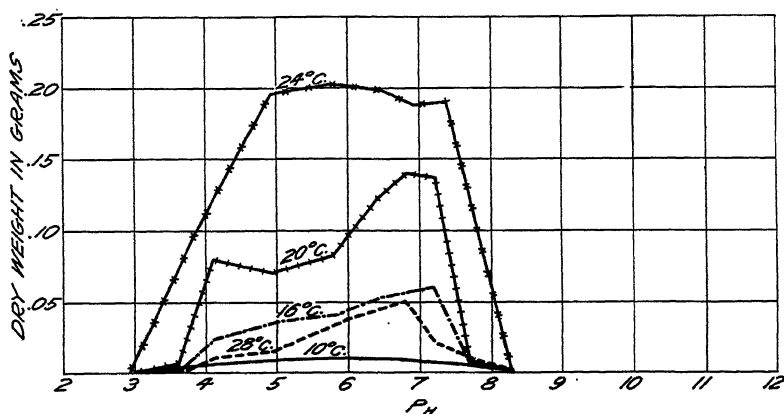


FIG. 4.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* in potato-dextrose decoction at different temperatures for a period of 12 days in diffused light.

A corn-meal decoction, as shown by Table 7, furnished growth at concentrations from P_H 3.6 to P_H 12.1. Two series of experiments were conducted in the dark for 12 days at $24^\circ C$. The first series produced only from one-third to one-fifth as much growth, on the average, as the second. Figure 3 contains the growth curve for the second series. Such uniform variation throughout the growth range might perhaps be explained on a basis of vitality of the culture which served for the inoculum. Similar variations have occurred for different series with a particular medium. A corn-meal decoction is a poorly buffered medium, and for this reason it is rather unsatisfactory for this type of experimentation.

TABLE 7.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, in corn-meal decoction at different initial P_H values in the dark for 12 days at $24^\circ C$.*

Hydrogen-ion concentration, P _H			Dry weight of fungous mat	Hydrogen-ion concentration, P _H			Dry weight of fungous mat
Initial	Final			Initial	Final		
	Control	Inocu- lated			Control	Inocu- lated	
			Gms.			Gm.	
3.2	3.0	3.0	0.0000	3.3		0.0000	
3.3	3.2	3.2	.0000	3.7	3.6	a F	
3.6	3.6	4.1	.0117	5.7	5.6	.1308	
4.3	5.6	5.8	.0316	7.1	6.0	.1432	
6.9	6.0	6.7	.0358	7.9		.1331	
8.1	7.9	7.3	.0277	8.1	7.5	.1192	
8.2	8.3	7.6	.0357	8.5		.1304	
8.7	8.4	7.9	.0383	8.8	8.4	.1469	
10.4	8.9	8.4	.0340	10.3	8.9	.1406	
11.5	9.3	9.0	.0217	11.0		.1263	
12.1	9.5	9.4	.0161	11.6	9.3	.1048	
12.2	9.6	9.6	.0000				

a F=trace of growth on edges of inoculum block.

Ophiobolus graminis grew feebly on the ordinary Czapek's solution and variably on Czapek's solution modified by the substitution of nucleic acid for sodium nitrate, as shown by Table 8. In the ordinary Czapek's solution at $24^\circ C$, growth occurred throughout the range P_H 4.5 to P_H 10.5. Greatest growth occurred at P_H 7.0 and at P_H 8.0, with an intervening minimum at P_H 7.6. In the nucleic-acid Czapek's solution, the reactions permitting growth ranged from P_H 3.6 to P_H 11.7, and the maximum growth of the fungus occurred in the cultures testing P_H 8.3. Reference to Figure 3 will reveal these relations. The hydrogen-ion concentration necessary to limit the growth on the alkaline side lay beyond the range of the experiment, i. e., P_H 11.7. Such also was the case with the corn-meal decoction. Growth was slightly better in the nucleic-acid Czapek medium than in the ordinary Czapek medium. In a preliminary series containing nucleic acid and adjusted to P_H 7.0, growth was highly stimulated. Definite and well-formed mats occurred in all of the cultures and the growth resembled that on potato-dextrose decoction.

TABLE 8.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, in ordinary and modified Czapek's solutions at different initial P_H values for various periods in diffused light and in the dark at 24° C.*

Media	Incubation	Hydrogen-ion concentration, P _H			Dry weight of fungous mat
		Initial	Final		
			Control	Inoc- ulated	
					Gm.
Czapek's solution.....	29 days in diffused light.	2.6	2.6	2.7	0.0000.
		3.0	3.0	3.0	0.0000.
		3.4	3.5	3.5	0.0000.
		4.5	4.5	3.9	0.0092.
		5.6	5.5	4.5	0.0051.
		6.6	6.4	6.5	0.0268.
		7.0	6.8	7.2	0.0674.
		7.6	7.6	6.2	0.0214.
		8.0	8.0	7.6	0.0546.
		10.5	9.0	9.0	0.0093.
		2.6	2.7	2.6	0.0000.
		3.6	3.0	3.6	0.0218.
		4.4	-----	4.4	0.0257.
		5.4	5.4	5.1	0.0251.
Czapek's solution (with nucleic acid).	12 days in dark.....	5.7	5.7	5.3	0.0298.
		6.6	6.4	6.4	0.0384.
		7.0	6.8	6.6	0.0527.
		7.9	7.2	6.8	0.0459.
		8.3	7.5	7.4	0.0836.
		9.4	8.6	8.2	0.0419.
		10.8	9.0	8.6	0.0381.
		11.4	9.1	9.1	0.0220.
		11.7	9.3	9.4	0.0166.
		3.6	3.4	-----	0.0000.
		4.7	3.9	-----	0.0138.
		5.5	4.9	5.2	0.0262.
		5.8	5.3	5.3	0.0301.
		6.5	6.0	5.6	0.0319.
Czapek's solution (cara- melized strongly).	41 days in dark.....	6.8	6.5	5.8	0.0293.
		7.3	6.8	6.5	0.0271.
		9.2	7.2	7.0	0.0271.
		9.6	7.4	7.1	0.0238.
		10.2	7.6	7.4	0.0204.
		10.7	8.7	8.2	0.0299.
		11.0	9.0	8.6	0.0298.
		3.6	-----	-----	No growth.
		3.8	-----	-----	Trace.
		5.3	-----	-----	Heavy mat.
		5.9	-----	-----	Do.
		6.8	-----	-----	Do.
		7.5	-----	-----	Do.
		7.9	-----	-----	Do.
Czapek's solution (cara- melized slightly).	12 days in dark.....	8.3	-----	-----	Do.
		9.4	-----	-----	Medium mat.
		9.9	-----	-----	Growth throughout, no mat.
		10.7	-----	-----	Slight growth.
		11.1	-----	-----	No growth.

It was repeatedly noticed that several flasks from each lot of Czapek's solution possessed a faint brownish tint. This condition occurred even though the acid and alkali were sterilized separately and subsequently added to the various cultures. The phenomenon of caramelization is extremely complicated and is understood only in a general way. In this connection, it must be remembered that the initial reaction of Czapek's solution generally tested P_H 4.6 and that the carbohydrate employed was sucrose. Such conditions are very conducive to caramelization. Heavy fungous mats, similar to those formed in potato-dextrose decoction, were produced in caramelized cultures of Czapek's solution as contrasted with the

small fungous colonies produced in the noncaramelized cultures. A preliminary series of experiments was conducted for a period of 12 days at 24° C. with caramelized Czapek's solution adjusted to various initial reactions. The results are presented in Table 8. Growth was obtained within the range of P_H 5.3 and P_H 10.7 with heavy fungous mats produced in cultures testing between P_H 5.3 and P_H 8.3 inclusive. In this very preliminary series no dry weights and no final hydrogen-ion concentrations of the substrate were determined. The curve for caramelized Czapek's solution in Figure 3 was approximated from the results of this series.

Incubated under similar conditions, very slight growth occurred in the strongly caramelized solutions throughout the entire range, i. e., P_H 4.7 to P_H 11.0, as shown by Table 8. This uniformly poor growth can not be attributed to the viability of the inoculum, inasmuch as the cultures were reinoculated 10 days after the first inoculation. The treatment given the solutions evidently was too drastic to permit growth of the fungus. In this case, it will be remembered that the concentrated nutrient solution (10X) was caramelized by autoclaving for 4 hours at a 15-pound pressure as compared with 2 hours at a 15-pound pressure in the other. These solutions, as determined by the intensity of the amber color, were caramelized to a much higher degree than those of the few slightly caramelized cultures appearing in the ordinary series, or of the slightly caramelized cultures in the preliminary series. Whereas a slightly caramelized Czapek's medium stimulated growth, a strongly caramelized Czapek's medium proved toxic to the fungus. A similar relationship was noted for varying degrees of caramelization in the alkaline cultures of potato-dextrose agar.

In the studies with *Ophiobolus graminis*, the final hydrogen-ion determinations showed that the fungus generally induced a shift in reaction toward alkalinity in a potato-dextrose medium, shifts toward acidity in Czapek's nucleic-acid and Czapek's ordinary media, and a shift toward neutrality in a corn-meal decoction. There appeared early in the work, however, indications that the changes in the liquid media did not always continue in their original direction.

For the studies of progressive growth, several series of experiments were conducted with potato-dextrose decoction adjusted to different initial reactions, namely, P_H 4.3, the most acid reaction allowing good growth; P_H 5.5, a potato-dextrose decoction without additions of either acid or alkali, and P_H 6.5, the reaction near the alkaline limit affording good growth. The necessary number of inoculated and uninoculated flasks were placed in the dark at 24° C. Three inoculated cultures and one uninoculated control were removed every two days. Dry weights of the fungous mats and the hydrogen-ion concentrations of inoculated and uninoculated media were determined at each periodic removal. The progressive relations for the growth of the fungus and for the changes in reaction of the medium are presented by data in Table 9, and by curves in Figures 5 and 6. At first, growth was more rapid in the nearly neutral cultures; later, it was greater in the more acid cultures. In general, growth increased with the incubation interval, and the greatest increase of growth occurred during the last two days of the experiment, namely, between 18 and 20 days.

TABLE 9.—The progressive growth of *Ophiobolus graminis*, and the progressive changes in reaction induced by growth, in potato-dextrose decoction in the dark for various periods at 24° C.

Age of cultures	Dry weight of material	Hydrogen-ion concentration, P_H		
		Initial	Final	
			Control	Inoculated
<i>Days</i>	<i>Gm.</i>			
2	0.0036	4.3	4.3	4.3
4	.0114	4.3	4.3	4.3
6	.0237	4.3	4.3	4.4
8	.0959	4.3	4.3	5.1
10	.1756	4.3	4.3	5.7
12	.1820	4.3	4.3	5.4
14	.2568	4.3	4.3	5.4
16	.2384	4.3	4.3	4.9
18	.2636	4.3	4.3	4.9
20	.5194	4.3	4.3	4.9
2	0.0019	5.5	5.5	5.5
4	.0151	5.5	5.5	5.5
6	.0236	5.5	5.5	5.7
8	.0859	5.5	5.5	6.7
10	.1478	5.5	5.5	7.8
12	.1908	5.5	5.5	6.9
14	.2079	5.5	5.5	6.8
16	.1522	5.5	5.5	5.7
18	.2340	5.5	5.5	5.5
20	.4340	5.5	5.5	4.9
2	0.0035	6.5	6.5	6.5
4	.0155	6.5	6.5	6.6
6	.0267	6.5	6.5	6.6
8	.1048	6.5	6.5	7.2
10	.1288	6.5	6.5	7.4
12	.1627	6.5	6.5	7.0
14	.1814	6.5	6.5	5.8
16	.1770	6.5	6.4	5.5
18	.2103	6.5	6.4	5.4
20	.3898	6.5	6.3	5.2

The changes in reaction of the potato-dextrose decoction during growth of the fungus were very definite and similar for each of the initial reactions, as shown by Table 9 and Figure 6. The uninoculated control cultures held their initial reaction throughout the 20 days of the experiment, and they will not be considered further. At P_H 4.3, the acidity of the medium decreased with increased growth until a value of P_H 5.7 was reached on the tenth day. After this date, even though growth continued to increase, the acidity increased until the final reaction tested P_H 4.9 for 20 days of fungous growth. A distinct reversion in reaction of the medium occurred on the tenth day, and the final reaction was six-tenths of an exponent less acid than that representing the initial reaction. Similar but greater changes were obtained at P_H 5.5. The reaction of the substrate progressively changed until a value of P_H 7.8 was reached on the tenth day. After this point, a reversion in reaction manifested itself, progressive changes occurred, and P_H 4.9 was the final reaction recorded. It is interesting to note that the cultures possessed a final value six-tenths of an exponent more acid than they did originally. Very similar changes were obtained for the cultures initially testing P_H 6.5. The changes increased successively until the tenth day was reached and a value of P_H 7.4 exhibited. After this time the changes proceeded

in the opposite direction and the final hydrogen-ion concentration was P_H 5.2. This value is actually one and three-tenths of an exponent more acid than originally. The greatest changes in reaction induced by growth occurred between the sixth and the sixteenth days of incubation and, of the three initial reactions employed, namely, P_H 4.3, P_H 5.5, and P_H 6.5, the greatest changes in reaction of the substrate occurred at P_H 5.5.

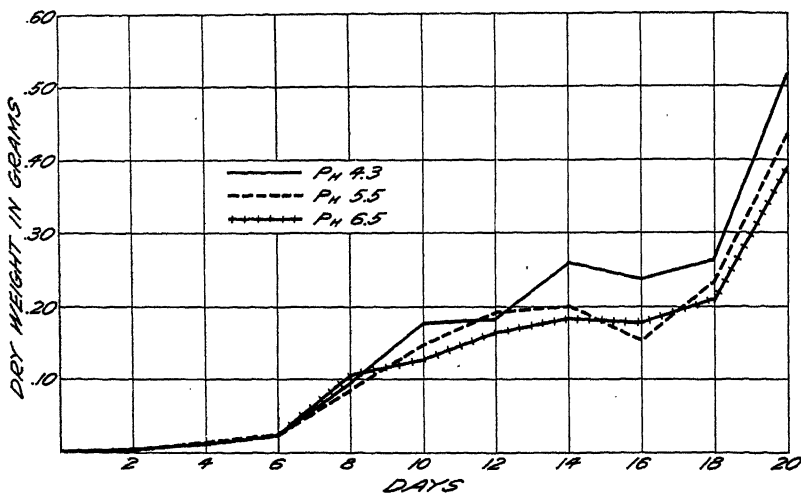


FIG. 5.—The growth of *Ophiobolus graminis* in potato-dextrose decoction of different initial P_H values for various periods in the dark at $24^{\circ}C$.

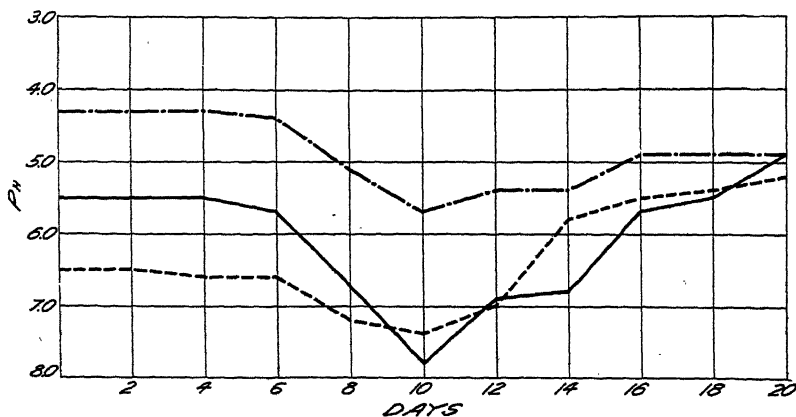


FIG. 6.—The changes in reaction of potato-dextrose decoction of different initial P_H values induced by the growth of *Ophiobolus graminis* for various periods in the dark at $24^{\circ}C$.

*Similar progressive series of experiments were conducted with Czapek's full nutrient solution modified by the substitution of nucleic acid for $NaNO_3$ and adjusted to initial values of P_H 4.9 and of P_H 8.3. The results are shown in Table 10 and in Figures 7 and 8. Very little growth was obtained at P_H 4.9, and it was more or less constant after an incubation of six days. Growth was much better at P_H 8.3, and increased rather uniformly with increase in

time interval. The reactions of the cultures at different ages showed reversion phenomena, but they were in the opposite direction to those occurring in potato-dextrose decoction. In the cultures initially testing P_H 4.9, the changes were relatively small. The acidity of the substrate increased with increase in age of the culture until a value of P_H 4.2 was reached on the fourteenth day. Two days later, the reaction became P_H 4.4, and remained constant throughout the duration of the experiment. Similar changes of

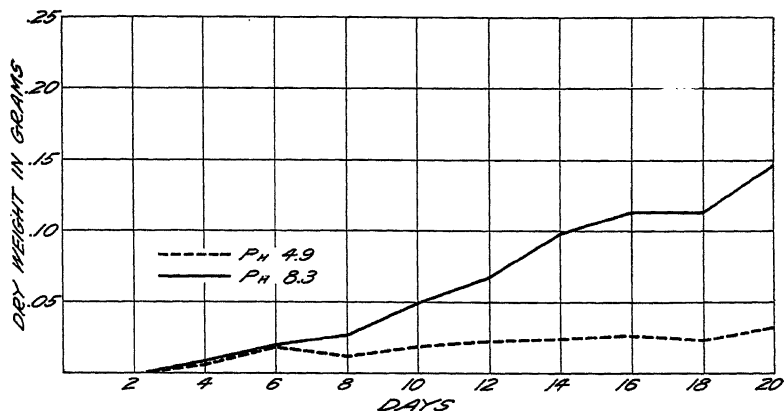


FIG. 7.—The growth of *Ophiobolus graminis* in Czapek's nucleic-acid solution of different initial P_H values for various periods in the dark at $24^\circ C$.

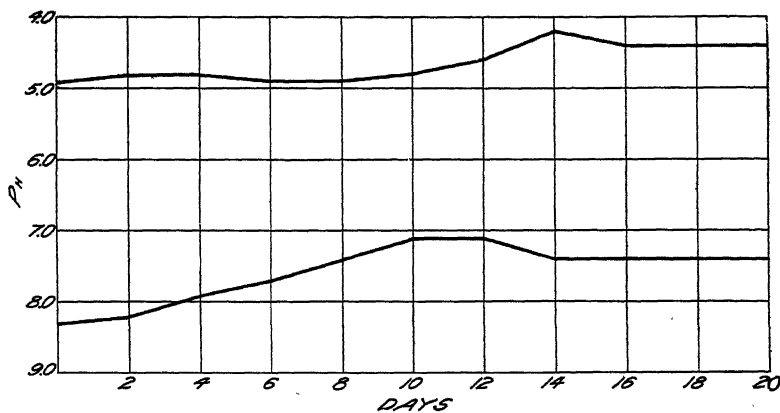


FIG. 8.—The changes in reaction of Czapek's nucleic-acid solution of different initial P_H values induced by the growth of *Ophiobolus graminis* for various periods in the dark at $24^\circ C$.

greater magnitude occurred in the cultures testing P_H 8.3. The alkalinity of the substrate decreased with increased period of incubation and attained its lowest value, namely, P_H 7.1 for both 10 and 12 days' growth. The reaction shifted to P_H 7.4 after two additional days of growth, and subsequent determinations proved it to be constant for the remainder of the experiment. The changes were first in the direction of the acid side and then in the direction of the alkaline side. Opposite shifts, on the other hand, appeared with potato-dextrose decoction. In the Czapek medium, the magnitude

of the second shift did not equal that of the initial shift. Such a relation held true for the potato-dextrose decoction only at an initial P_H 4.9, whereas at initial reactions of P_H 5.5 and P_H 6.5 the reversion shifts were greater than the initial shifts.

TABLE 10.—*The progressive growth of Ophiobolus graminis, and the progressive changes in reaction induced by growth, in Czapek's nucleic-acid solution in the dark for various periods at 24° C.*

Age of cultures	Dry weight of material	Hydrogen-ion concentration, P_H		
		Initial	Final	
			Control	Inoculated
<i>Days</i>	<i>Gm.</i>			
2	° Tr.	4.9	4.9	4.8
4	0.0077	4.9	4.8	4.8
6	.0218	4.9	4.9	4.9
8	.0132	4.9	4.9	4.9
10	.0196	4.9	4.8	4.8
12	.0230	4.9	4.8	4.6
14	.0252	4.9	4.8	4.2
16	.0274	4.9	4.8	4.4
18	.0229	4.9	4.8	4.4
20	.0318	4.9	4.8	4.4
2	Tr.	8.3	8.3	8.2
4	.0106	8.3	8.3	7.9
6	.0219	8.3	8.3	7.7
8	.0285	8.3	8.4	7.4
10	.0503	8.3	8.3	7.1
12	.0888	8.3	8.3	7.1
14*	.0982	8.3	8.4	7.4
16	.1134	8.3	8.3	7.4
18	.1124	8.3	8.3	7.4
20	.1458	8.3	8.3	7.4

* Tr. = trace of growth.

In order that it might be determined whether growth had merely been inhibited or the fungus had actually been killed in the extremely acid and alkaline cultures not showing growth, the inoculum blocks were transferred from such cultures to potato-dextrose agar slants at the termination of several experiments with potato-dextrose decoction and with Czapek's solution. In potato-dextrose decoction, it will be remembered, growth occurred throughout the range P_H 3.9 to P_H 7.7, and no growth occurred in the acid cultures testing P_H 2.4, 2.9, and 3.6, or in the alkaline cultures testing P_H 8.4, 8.6, 8.8, and 9.5. The results obtained from the transfers show that the fungus was killed at the reactions P_H 2.4 and 9.5 and that it was still alive at the reactions P_H 2.9, 3.6, 8.4, 8.6, and 8.8 even though no perceptible growth had occurred at these reactions for a period of 12 days. Very consistent results were obtained at all the reactions except P_H 2.9, where probably not more than 50 per cent of the transfers grew. This indicated that such a reaction was very close to the critical one for the fungus in this medium. Somewhat similar but variable results were obtained with the transfers from Czapek's solution.

When acid or alkali in sufficient quantities to neutralize the acid or alkali originally employed for the adjustment of the reactions were added to the cultures of potato-dextrose decoction not showing

growth, no perceptible growth was ever exhibited. Subsequent transfers of the inoculum blocks were not made, but it would seem that nutritional disturbances produced by the second addition of either acid or alkali were responsible for either inhibition of growth or death of the fungus.

EFFECT OF LENGTH OF INCUBATION PERIOD

The correlation between the growth of *Ophiobolus graminis* and the hydrogen-ion concentration of the medium is influenced to a considerable degree under certain conditions by the length of the incubation period. In potato-dextrose decoction, growth generally occurred first and most rapidly at or near neutrality. However, with continued incubation, the rate of growth and the total quantity of growth in the acid cultures surpassed that in the approximately neutral ones. Different types of growth curves, therefore, were obtained when cultures were incubated under the same conditions for various periods. The growth curves in Figure 2 for two potato-dextrose decoction series at 24° C., one for a period of 8 days and the other for a period of 12 days, illustrate this point.

For the 8-day series, the growth curve gradually rose from P_H 3.6, reached a distinct maximum at P_H 7.2, and declined rapidly to P_H 7.7. The 12-day series, on the other hand, produced a rather flat type of growth curve with maximum growth occurring rather uniformly throughout a zone of reactions, namely, P_H 4.9 to P_H 7.4. This curve obtained for 8 days' growth at 24° C. is almost identical with the one obtained for 12 days' growth at 16°, and very similar to those obtained for 12 days' growth at 20° and 28°. At 10° growth was very small and more or less uniform throughout the growth range for a period of 12 days. An additional 15-day period of incubation at this temperature gave a bimodal growth curve with the primary maximum occurring at P_H 4.9 and the secondary one near P_H 7.0. Growth occurred from P_H 3.9 to P_H 8.4 and the range was slightly wider than usual, the alkaline limit having shifted a few tenths of a P_H toward greater alkalinity. The length of time necessary for the fungus to stale potato-dextrose decoction, 50 c. c. portions per culture, and thus cease growing has not been determined. Experiments were conducted over a period of 20 days and cultures were removed every two days. Growth continued throughout the entire period and occurred most rapidly for each of the three initial reactions near the end of the experiment. The data for potato-dextrose decoction are assembled in Tables 5, 6, and 9, and are graphically represented by curves in Figures 2, 3, 4, and 5.

Czapek's nutrient solution, modified by the substitution of nucleic acid for sodium nitrate, gave different results from those furnished by potato-dextrose decoction. In the acid cultures, feeble growth appeared early, but it never increased to any perceptible degree after the sixth day. Good growth, on the other hand, was obtained in the alkaline cultures and it increased more or less uniformly with increased periods of incubation. These results are included in Table 10 and serve as a basis for the curves in Figure 7.

With the corresponding solid media, growth occurred first and most rapidly at certain initial reactions, depending upon the medium, and the relations which were established early generally persisted throughout the course of the experiment. The fungous colonies were meas-

ured daily and very consistent data were obtained for the several media employed. At the optimum reactions, a period of 6 to 7 days was generally necessary to enable the fungous colonies entirely to cover the plates. The cultures were allowed to incubate over a period of 12 to 15 days and, while it is not possible to say anything definitely about the later growth at the favorable reactions, accurate growth data were obtained for the other reactions until the fungous colonies completely covered the plates.

Simultaneous with the studies on progressive growth in potato-dextrose decoction and Czapek's modified solution, progressive changes in reactions of the substrate induced by growth of the fungus were likewise followed. The occurrences of progressive and reversible shifts in reaction during the growth of this organism detract more or less from any significance that might be attached to the final hydrogen determinations and emphasize the importance of following such changes in reaction of the medium during growth.

EFFECT OF TEMPERATURE AND LIGHT

Like the chemical composition and the physical nature of the medium, temperature influenced the growth of the fungus, as related to hydrogen-ion concentration of the medium. Within the limits of the experiments, temperature did not materially alter the P_H range within which growth occurred, but it did influence the magnitude of growth at various initial reactions. Inasmuch as potato-dextrose decoction produced the best fungous growth, the temperature studies were confined principally to this medium. A few experiments, however, were conducted with the Czapek's synthetic solution, but growth in this medium was so poor that the results are not satisfactory.

Incubated for a period of 12 days in diffused light, the fungus grew best in potato-dextrose decoction at 24° C., followed in order by 20°, 16°, 28°, and 10°. The data presented in Table 6 and represented graphically in Figure 4 reveal these relations. At the optimum temperature, the growth range extended from P_H 3.6 to P_H 7.7, with best growth occurring more or less uniformly between P_H 4.9 and P_H 7.4. The growth range was practically the same for each of the other temperatures. The alkaline limit remained the same, and the acid limit at 28° and 16°, shifted several tenths of an exponent toward neutrality. At 24° growth in the extreme cultures was good, but at the other temperatures it was very small, in fact almost immeasurable in certain instances. Maximum growth of the fungus occurred in the form of a peak at or near P_H 7.0 for temperatures of 20°, 16°, and 28°, as contrasted with a broad zone from P_H 4.9 to P_H 7.4 for 24°. Heavy fungous mats were produced in all of the cultures at 24° and in those with favorable reactions at 20°. Growth was much less at 16° and 28°, and there were either very thin fungous mats or no mats at all. At 10°, mycelial growth was exceedingly slight and uniform, an interval of seven days being necessary before a trace of growth could be detected around the inoculum block. The final colonies were thin, flat, grayish, and filmlike.

Light is frequently regarded as an important factor for the fruiting of certain fungi, but neither perithecia nor indications of such appeared during the course of the experiments reported in this paper. Davis (4) has stated that diffused light slightly retarded mycelial development of *Ophiobolus graminis* in pure culture. In our studies,

however, diffused light did not seem to exert any appreciable effect on the relation of vegetative growth to the reaction of the medium.

Two series of experiments were conducted with potato-dextrose decoction at 24° C., one in the dark and one in diffused light. The growth curves, Figures 3 and 4, developed from the two experiments are very similar. There was a slightly greater tendency for a bimodal-growth curve in the case of the series incubated in the dark than in the one in the light. The variations in the two growth curves, however, were not very great and the P_H growth ranges were the same in both cases. The fungous mats obtained from the series grown under diffused light were thick, heavy, and substantial. They adhered strongly to the sides of the flasks and always remained in perfect form at the surface of the liquid medium. On the other hand, the fungous mats obtained from the series incubated in the dark were equally as thick and heavy, but were less firmly attached to the sides of the flasks. These mats generally became either completely or incompletely detached from the flasks and consequently appeared partially or totally submerged in the culture solutions. More or less consistent results were obtained for potato-dextrose decoction in the light and in the dark at 10°, and for Czapek's unmodified solution in the light and in the dark at 24° and 28°. In view of these consistencies, the tabulated results of the experiments for each series in the light and in the dark have not been included.

DISCUSSION

The results obtained by the writers show that the influence of the hydrogen-ion concentration on the vegetative growth of *Ophiobolus graminis* in pure culture is variable, and depends to a large extent on the chemical composition and the physical nature of the medium. The P_H ranges for fungous growth may be either wide or narrow, more generally the former, and the growth curves may be either monomodal or bimodal. Furthermore, the optimum P_H for fungous growth may fall either in the distinctly acid or the alkaline regions, or it may span the intervening neutral zone. It appears from these facts, therefore, that the general conclusions drawn by Kirby (7, 8) and Davis (4), regarding the influence of the hydrogen-ion concentration on the growth of the parasite in pure culture, are valid only within the limits of their experiments.

Brittlebank (2), who has investigated the take-all problem for a number of years in Australia, frankly admits that very little is known concerning the influence of soil reaction on the development of the disease. From the results obtained by him in one year's study on the permanent test plats, it appears that where lime in combination with other fertilizers had been applied on alternate years during a period of six years, the disease was far more prevalent than where lime had been omitted. This correlation refers more to the percentage of diseased plants than to the severity of the disease. Brittlebank says: "It would appear, therefore, that an alkaline soil is favorable to the development of the disease, but it should be again remarked, this judgment is from one year's data only." Another statement under the same heading, namely, "it is not known whether the fungus favors an acid or an alkaline soil, or one rich in organic matter," clearly indicates that Brittlebank was not yet ready to draw a definite conclusion on the subject.

Kirby (8) also has studied the development of the take-all disease under greenhouse and field conditions, and he recommends the adjustment of soil reaction as one of the three possible methods for protection against the disease. In the first greenhouse experiment, he conducted inoculation experiments in 5-inch pots filled with soil to which chemicals in varying quantities had been added. Very abnormal growth occurred in all cases, and a few plants reached a state of maturity only in the case of the sulphur-treated soil. No determinations of soil reaction were made, but nevertheless he endeavors to correlate the severity of the disease with an alkaline reaction. However, his figures actually show that, while the least infection did appear in the sulphur series, a higher percentage of plants were killed on the untreated soil than on the limed soil. In another greenhouse series with soil adjusted to various reactions by regulated additions of sulphuric acid or sodium hydroxide, all the plants growing in soil ranging from P_H 5.2 to P_H 8.8 were severely attacked by the fungus and ultimately killed by the disease. Soil reactions of P_H 2.3, 3.0, and 4.0 seemed to control the disease, but these soil reactions were unfavorable to the development of the host. The plants remained alive during the course of the experiment, but they never attained a height greater than a few inches. Under the condition of the experiment, therefore, Kirby concluded that no degree of active acidity which permitted normal growth and maturity of the plants controlled the disease.

Kirby (8) has studied the disease response to sulphur, sulphuric acid, and lime when added to the soil under field conditions in New York State. He obtained very high percentages of diseased plants in the sulphuric acid, lime, and untreated plats, and a somewhat lower but moderately high percentage in the sulphur plat. No hydrogen-ion determinations were made on the soils in question. While the addition of lime increased and that of acid decreased the percentages of badly diseased plants, it appears that no striking correlation existed between the total percentages of diseased plants and the addition of alkaline or acid substances. The disease was not effectively controlled by the additions of sulphuric acid in various proportions to the soil, and it was only partially controlled by the additions of sulphur. Moreover, in the sulphur series, the average weight of grain per plant for the healthy plants and for practically all of the diseased plants was strikingly small. Such low yields of grain on very acid soils certainly inhibit any profitable control of the disease by the applications of acid or acid-forming substances to the soil.

The results obtained by the writers show that the nutritional and physical natures of the medium, irrespective of other factors, greatly modify the influence of the hydrogen and hydroxyl ions on the growth of the fungus in pure culture. The question naturally arises as to whether soil fertility and soil type modify the influence of the soil reactions on the growth of the fungus and the occurrence of the disease in the field. This question can not be answered definitely at present, but in the light of the diverse results obtained by the writers, it will be rather surprising if variations in soil type and fertility are not found to produce similar effects. Furthermore, it appears that the results in the control of the disease under greenhouse and field conditions by the addition of acid or alkaline substances have been vari-

able and more or less unsatisfactory from the standpoint of host development. In view of these facts, therefore, it would seem that the control of take-all by the mere adjustment of soil reaction will be uncertain.

SUMMARY

In general, *Ophiobolus graminis* grew well in pure culture on solid and liquid media over a wide range of active acidity and alkalinity. The P_H range and the P_H optimum for growth were variables, depending upon the physical and chemical nature of the medium and the environmental factors.

Comparing the different solid media, the rates of growth and the total quantities of growth were more or less uniform within the favorable P_H zones. On the corresponding liquid media, the best growth occurred consistently in potato-dextrose decoction, followed in order by Czapek's solution (slightly caramelized), corn-meal decoction, and Czapek's solution (nucleic acid).

Temperature influenced the optimum reaction for fungous growth in potato-dextrose decoction, but did not materially influence the P_H range through which growth occurred. The optimum temperature was not altered by variations in the hydrogen-ion concentration.

The length of the period of growth influenced the P_H optimum for fungous growth in potato-dextrose decoction but did not appreciably modify the P_H growth range. No shifts in the optimum reaction were noted for the solid media employed.

Neither diffused light nor total darkness appreciably affected the P_H optimum or the P_H range for fungous growth. However, a slightly greater tendency for a bimodal-growth curve occurred in the cultures in the dark than in those in the light.

While the hydrogen ion generally proved to be more toxic to the growth of the fungus than the hydroxyl ion, the toxicity of each was variable, depending upon the medium. It was only in a potato-dextrose decoction that the fungus was particularly sensitive to a condition of active alkalinity.

The reaction of the substrate sufficient to cause death of the fungus was several tenths of a P_H more acid or more alkaline than that sufficient to cause inhibition of growth.

Slight caramelization of Czapek's nutrient solution stimulated growth to a great degree, but severe caramelization proved toxic to the fungus. Similar relations were noted in alkaline potato-dextrose agar.

Progressive and reversible shifts in the reaction of the media were induced by the growth of the fungus. The direction and the magnitude of these shifts varied with the temperature, the duration of the growth interval, the initial reaction, and the chemical and physical natures of the media.

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EXPERIMENTAL DISTURBANCES IN THE MILK SECRETION OF THE COW¹

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INTRODUCTION

The three major hypotheses that have been brought forth to account for the processes involved in the secretion of milk and its constituents are: (1) That the secretory cells themselves break loose, become disintegrated, and set free their contents in the alveoli; (2) that the portion of the secretory cell next to the alveoli—that is, the free end—after becoming loaded with secretory products, breaks loose from the basal portion, becomes disintegrated, and sets free its contents in the alveoli; (3) that the secretory cells discharge their secretory products into the alveoli, as do the salivary glands, without themselves becoming detached or destroyed.

The third of these hypotheses seems to the writer the most reasonable, and in the following paper experimental data are presented which support this view.

Eckles and Shaw (3),³ studying the variations in the composition of milk from the individual cow, found that normally the percentage of lactose, ash, and protein content fluctuates only slightly from one milking to another, whereas the percentage of fat content is quite variable. Jackson and Rothera (5) found that this normally slight variability in the percentage of lactose and ash content of cow's milk could be disturbed by returning a portion of milk to the udder after it was milked dry. The percentage of lactose in the milk produced at the milking following the return of milk was greatly decreased and the percentage of ash content (soluble ash) similarly increased. During the subsequent milkings both constituents gradually returned to their original condition. The freezing point of the milk produced both before and after the return of milk was practically the same, showing that there was a compensatory inverse relation between the percentage of lactose and soluble ash in the milk which tended to maintain a constant freezing point.

Owing to this very peculiar condition caused by the return of milk to the udder, a similar experiment was performed for the purpose of possibly securing an insight into the mode of milk secretion.

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² The author acknowledges his indebtedness to W. L. Gaines, M. J. Prucha, and J. M. Brannon for their kind assistance and suggestions during the progress of this investigation.

³ Reference is made by number (italic) to "Literature cited," p. 885.

OUTLINE OF EXPERIMENT

The experiment consisted of seven trials and involved the use of a purebred Jersey cow milked twice a day, and in the seventh month of her lactation. In trials 1 to 4 milk was returned to a quarter of the udder after it was milked dry. In trials 5 and 6 an isotonic salt solution was injected into a quarter of the udder after it was milked dry, and in trial 7 an 8.8 per cent solution of lactose, also isotonic, was similarly injected. Each trial was divided into two periods, one preliminary to the return of milk or the injection of solutions and one subsequent to it. In all the trials each quarter of the udder was milked separately and the milk produced was weighed and sampled separately. The sample of milk from each quarter during the preliminary and subsequent periods was analyzed for the percentages of fat, lactose, and total solids. It was impossible in most cases to make a complete analysis including the determination of protein and ash; hence these two were determined by difference, and are reported as protein+ash. In trials 6 and 9, however, a complete analysis was made.

The method of returning milk or injecting solutions to a quarter of the udder after it had been milked dry was very simple and apparently did not produce any pain, for the cow showed no signs of excitement. The usual simple method of injecting a teat canula into the teat of the udder was followed. To the canula was attached a piece of rubber tubing into the other end of which the tip of a pipette was inserted, the injection thus being easily accomplished. When milk was returned the udder was washed with warm water just before milking and the milk was received in sterilized cans. The milk returned to a quarter was the same milk that had been drawn from it. The milk was returned immediately after milking, so that its temperature was practically the same as when it was drawn. When salt or lactose solutions were used, they were sterilized in an autoclave and cooled to body temperature before being injected into the udder. An examination of the milk returned showed in every case that it was practically free from bacteria.

EXPERIMENTAL RESULTS

EFFECT OF RETURNING MILK TO THE UDDER

The four trials in which milk was returned to a quarter of the udder gave similar results, all of which confirm the findings of Jackson and Rothera (5) with respect to the variability in the percentage of lactose content of the milk produced. The results from two of these trials are reported graphically in Figures 1 and 2. A consideration of the curves in these figures brings out the following facts with respect to the variability in the percentage content of the milk produced during both the preliminary and subsequent periods.

The percentage of lactose and the percentage of protein+ash are not extremely variable from one milking to another during the preliminary periods or under normal conditions. The percentage of fat, on the other hand, is quite variable from one milking to another under normal conditions. Of course, these percentages are for milk

taken from only one quarter of the udder. This fact may account for the wide variations in the percentage of fat content, but at the same time it would tend to emphasize the lack of variability in the

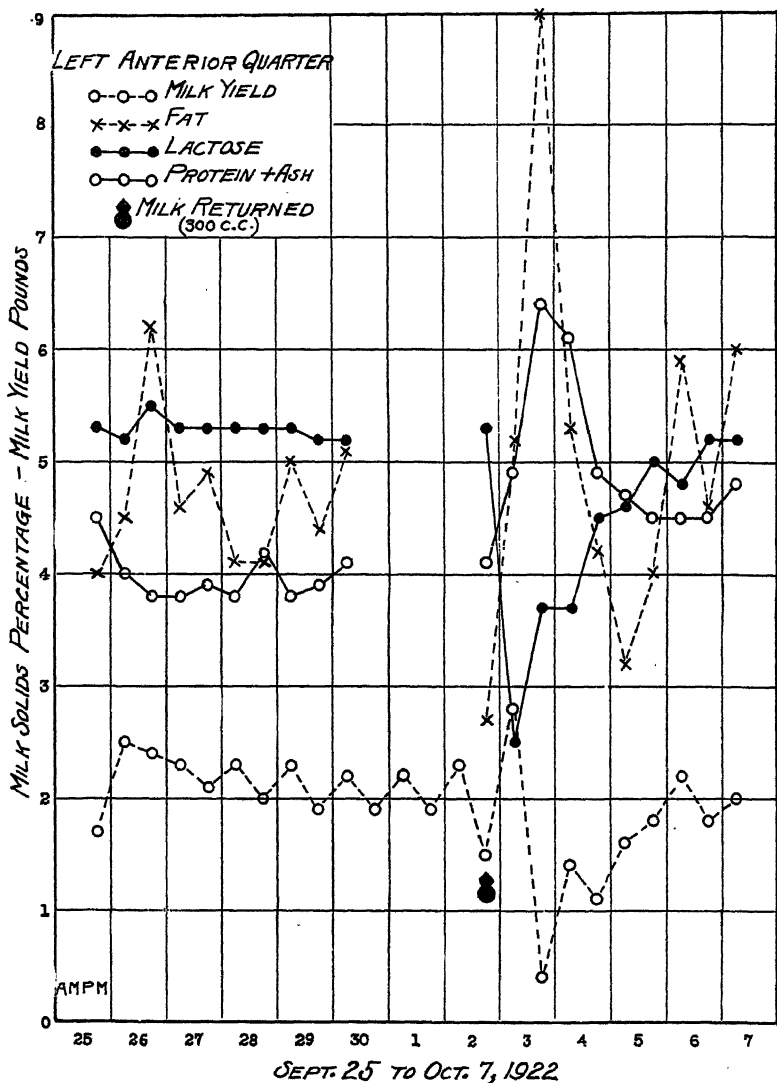


FIG. 1.—Yield of milk and percentage content of fat, lactose, and protein+ash in milkings before and after the return of 300 c. c. of milk to the left anterior quarter of the udder

percentage of lactose and of protein+ash. The variation in the composition of the milk produced during the preliminary period substantiates the findings of Eckles and Shaw (3), although the milk comes from only one-quarter and not from the whole udder.

During the milkings subsequent to the return of milk to the udder the percentage content of the milk produced is markedly disturbed. The percentage of lactose is at first greatly decreased and then gradually returns to its normal condition. The percentage of protein+ash increases rather markedly for the first two or three milkings and then gradually returns to its normal condition. The

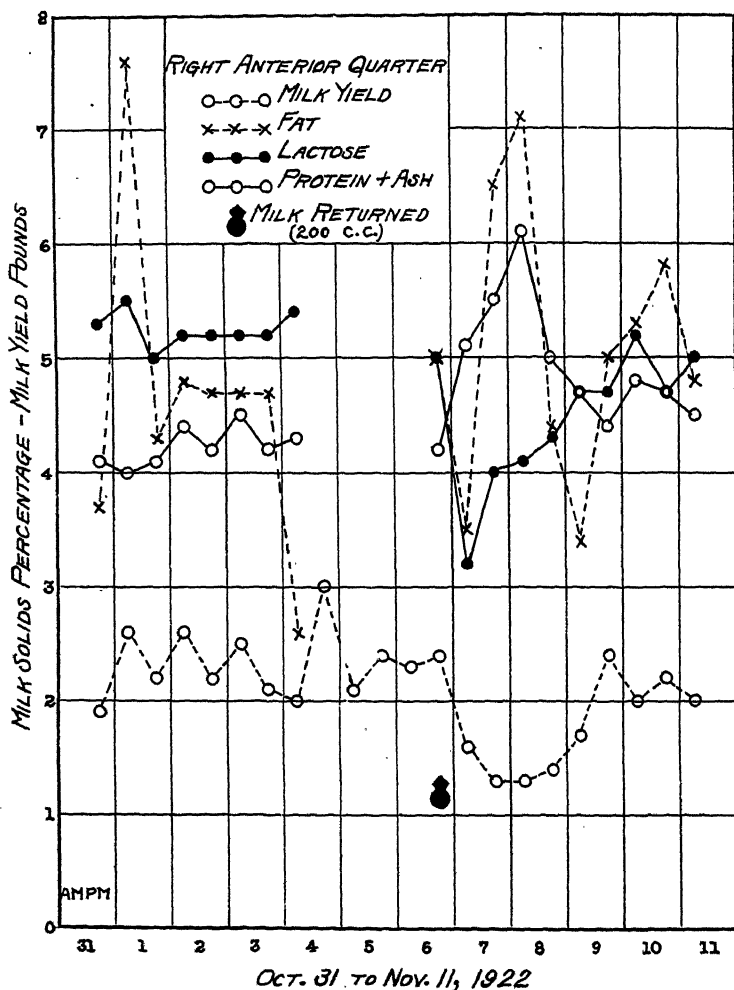


FIG. 2.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the return of 200 c. c. of milk to the right anterior quarter of the udder

percentage of fat content increases markedly during the second or third milking and then becomes quite variable. The percentage of fat content is quite variable under normal conditions, hence the increase after the return of milk to the udder may not at first seem significant. However, this increase was always consistent, and when solutions instead of milk were injected the percentage of fat content

increased beyond any conceivable variability under normal conditions. The freezing point of the milk produced during the preliminary and subsequent periods was not determined for every sample in these trials. Those that were determined, however, indicated that it changed very little even when the percentage content was greatly disturbed.

During the first two trials the milk produced by the quarters of the udder other than the experimental quarter was also analyzed. The analysis showed that the return of milk to one quarter did not affect the composition of the milk produced by the other quarters.

Considering the variability in the percentage content of the milk produced both before and after the return of milk to the udder, it is obvious that a slight deviation in the percentage of lactose or the percentage of protein + ash is far more significant than a somewhat larger deviation in the percentage of fat.

The degree of disturbance in the percentage content of the milk produced is dependent upon the quantity of milk returned in proportion to the yield of the quarter. The yields of the quarters represented in Figures 1 and 2 are nearly equal. Three hundred c. c. of milk was returned to the quarter represented in Figure 1 and 200 c. c. was returned to the quarter represented in Figure 2. The degree of disturbance in the percentage content of the milk produced is greater in Figure 1.

EFFECT OF INJECTING SALT AND LACTOSE SOLUTIONS INTO THE UDDER

In seeking a reason for this disturbance in the composition of the milk it was decided to inject the udder with solutions instead of milk, thinking that possibly such a procedure would give some clue to the cause of the disturbance. Accordingly 300 c. c. of an isotonic salt solution, consisting of the salts found in milk (sodium and potassium chloride and sodium and potassium citrate) was injected into a quarter of the udder after it was milked dry. The results from this the fifth trial are reported graphically in Figure 3.

From a consideration of the curves in this figure it is quite surprising to see that the injection of an isotonic salt solution produced a disturbance in the milk constituents similar to that produced by the return of milk to the udder. The effect upon the percentage of fat is very pronounced, raising it greatly above any variation that could be expected under normal conditions.

Trials 6 and 7 were run together and consisted of the injection of 300 c. c. of an isotonic salt solution to one quarter and 300 c. c. of an isotonic (8.8 per cent) lactose solution to another quarter. In these trials a complete chemical analysis was made of the milk produced at the milking done at the time of the injection and during part of the subsequent milkings. The results from these two trials are reported in Tables 1 and 2.

TABLE 1.—*Effect on the yield, composition, freezing point, and specific gravity of milk produced by the injection of an isosmotic salt solution into the right posterior quarter of udder, milked twice a day, December 18 to December 27, 1922*

Milkings	Milk yield	Fat	Lactose	Protein + ash	Protein	Ash	Freezing point	Specific gravity
Preliminary milkings:	<i>Lbs.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>° C.</i>	
A. m.-----	2.2	5.4	4.6	^a 4.8	-----	-----	-----	1.0328
P. m.-----	2.3	5.2	4.8	^a 5.3	-----	-----	-----	1.0355
Milking at time of injection of 300 c. c. of isosmotic salt solution, p. m.-----	2.1	3.4	4.7	4.18	3.40	0.78	-0.547	1.0347
	2.2	3.0	2.9	4.50	3.70	.80	-.548	1.0281
	.5	8.2	3.5	6.16	5.26	.90	-.559	1.0320
	1.7	10.0	3.7	6.34	5.50	.84	-.560	1.0326
	1.4	6.9	4.1	5.15	4.33	.82	-.545	1.0309
Subsequent milkings ^b -----	1.2	3.6	4.2	4.53	3.70	.83	-.540	1.0330
	2.2	3.3	4.0	4.50	3.70	.80	-.545	1.0319
	2.2	4.4	4.2	^a 4.6	-----	-----	-----	1.0311
	1.3	3.2	4.4	^a 4.8	-----	-----	-----	1.0334
	1.9	4.3	4.3	^a 4.9	-----	-----	-----	1.0328

^a Determined by difference.

^b Commencing with the first one, which was done in the morning, subsequent milkings were done alternately in the evenings and mornings.

TABLE 2.—*Effect on the yield, composition, freezing point, and specific gravity of milk produced by the injection of a lactose solution into the left posterior quarter of udder, milked twice a day, December 18 to December 27, 1922*

Milkings	Milk yield	Fat	Lactose	Protein ash	Protein	Ash	Freezing point	Specific gravity
Preliminary milkings:	<i>Lbs.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>° C.</i>	
P. m.-----	2.1	5.2	5.0	^a 4.5	-----	-----	-----	1.0334
A. m.-----	1.9	4.0	5.2	^a 4.8	-----	-----	-----	1.0361
Milking at time of injection of 300 c. c. of lactose solution, p. m.-----	1.8	2.5	5.0	4.53	3.80	0.73	-0.549	1.0373
	1.2	4.0	3.3	3.63	3.00	.63	-.550	1.0261
	.2	9.1	2.4	6.32	5.42	.90	-.550	1.0240
	1.0	12.0	2.9	7.54	6.66	.88	-.570	1.0312
	1.0	8.7	3.5	5.49	4.61	.88	-.532	1.0300
Subsequent milkings ^b -----	1.3	4.9	4.0	4.38	3.56	.82	-.547	1.0320
	1.5	2.9	4.1	4.35	3.55	.80	-.542	1.0307
	2.2	3.6	4.2	^a 4.4	-----	-----	-----	1.0311
	1.2	2.9	4.4	^a 4.5	-----	-----	-----	1.0326
	1.5	3.5	4.5	^a 4.7	-----	-----	-----	1.0334

^a Determined by difference.

^b Commencing with the first one, which was done in the morning, subsequent milkings were done alternately in the evenings and mornings.

The variability in the percentage contents reported in Tables 1 and 2 show that with few exceptions the results from these trials are similar to those secured in the other trials. The percentage of fat content, like that in trial 5, is increased far beyond any expected deviation under normal conditions. The percentage of ash and the percentage of protein both increased after the solutions were injected into the udder. Their sum, the percentage of protein+ash, showed variability similar to that found in the other trials. On the first milking after the injection of the lactose solution the percentage of lactose was not as low as on the second milking. In all of the other trials the percentage of lactose was lowest on the first milking after the return of milk or the injection of the salt solution. This exception is due to the fact that a large quantity of lactose was injected into the quarter in the 8.8 per cent solution. The actual quantity of lactose produced at this milking was only 65.8 per cent of the quantity introduced into the quarter in the 300 c. c. of

the 8.8 per cent solution. It will also be noticed in Tables 1 and 2 that the freezing point of the milk changed only very slightly during the subsequent milkings.

EFFECT OF INJECTING DISTILLED WATER INTO THE UDDER

It was next planned to inject 300 c. c. of distilled water into a quarter of the udder after it was milked dry, but unfortunately the

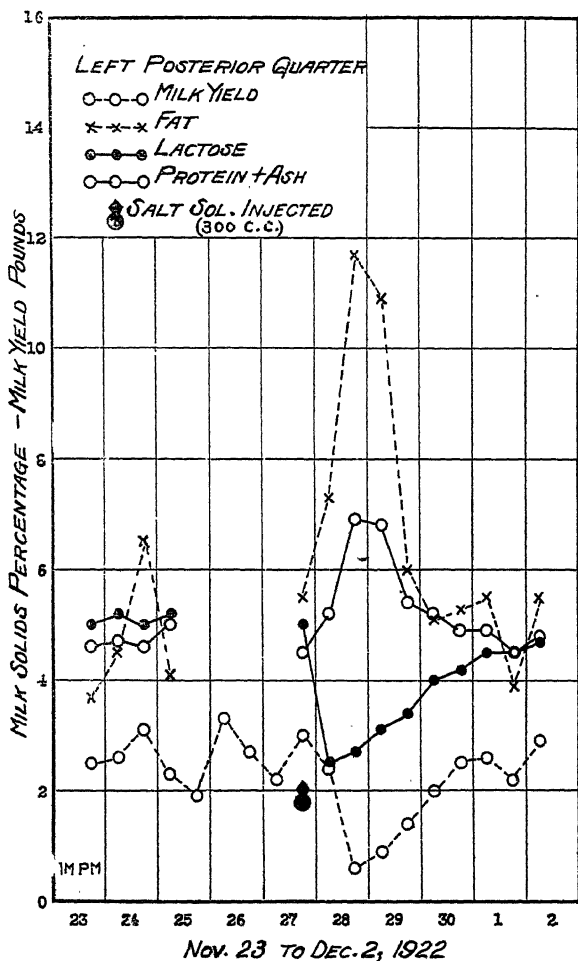


FIG. 3.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the injection of 300 c. c. of a salt solution into the left posterior quarter of the udder

experiment had to be discontinued. However, it has since been resumed, and sterile distilled water was injected into two quarters of the udder of a cow producing approximately the same quantity of milk as the cow used in the first part of the experiment. The method of procedure followed in injecting distilled water into the

udder was the same as that used in injecting salt and lactose solutions.

The results obtained in these two trials are graphically reported in Figure 4. In the milkings subsequent to the injection of 300 c. c. of distilled water only a very slight tendency was noted toward an

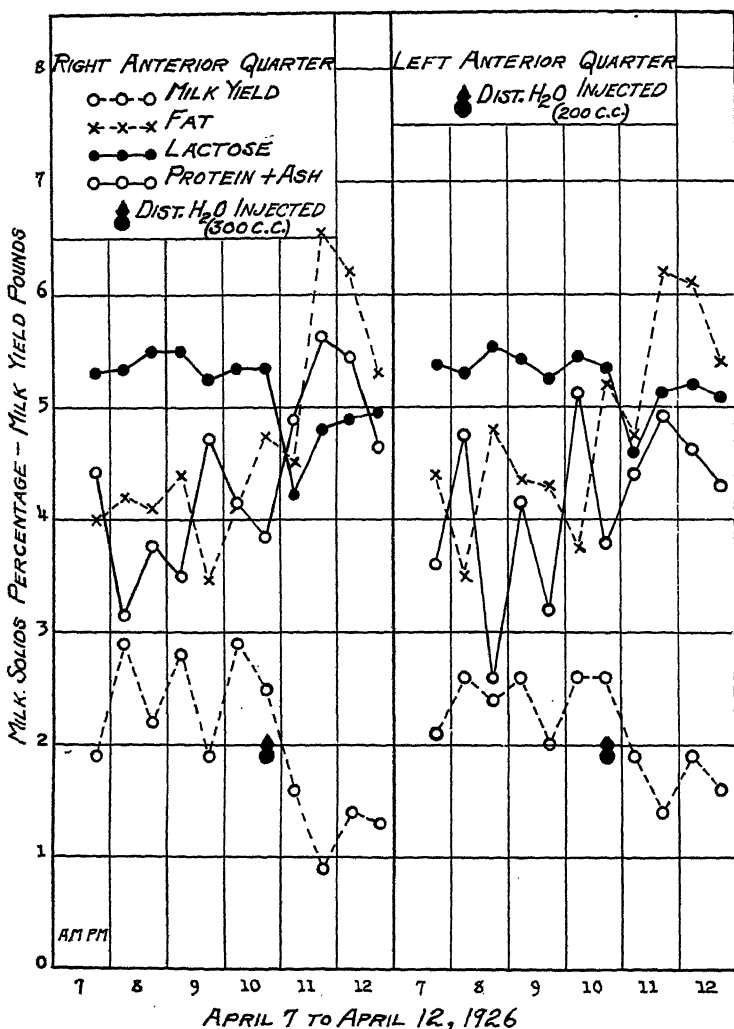


FIG. 4.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the injection of 300 c. c. of distilled water into the right anterior quarter, and 200 c. c. of distilled water into the left anterior quarter of the udder

abnormal variation in the percentage content of the milk such as was produced by returning 300 c. c. of milk or injecting salt or lactose solutions. In the milkings subsequent to the injection of 200 c. c. of distilled water there was likewise no significant abnormal variation in the percentage content of the milk. Considering the results of

these two trials, it seems as though there must be some factor other than the mere physical effect of the liquid which brings about the marked abnormal variations in the percentage content of the milk noted in the earlier experiments. This point will be further discussed later on.

EFFECT OF LEAVING MILK WITHIN THE UDDER

After the completion of the experiment described above a somewhat similar experiment was undertaken (2). In this test a study was made of the effect of a partial milking of the udder on the composition of the milk produced during the subsequent milkings.

The results from all of the trials in this test, wherein one-fourth, and then one-half, of the average daily evening production was left within the udder, indicate that there is very little similarity, if any, in the effect produced by leaving a portion of milk within the udder and returning a portion to the udder. The curves in Figure 5 illustrate the variations in the percentage content of the milk as a result of leaving one-half of the average daily evening production within the udder. The only percentage that seems to be affected to any extent is that of fat content.

DISCUSSION

Apparently the returning of a portion of milk to the udder after it is milked dry has an effect upon the secretive tissues of the udder altogether different from that produced by leaving a portion of milk within it. The writer can give no exact explanation for this difference. However, a description of some of the possible changes which may be taking place within the secretive tissues as a result of these manipulations of the udder may throw some light upon the subject.

A description of the processes involved in secretion is very precisely given by Bayliss (*1, p. 348*) in the following quotation:

On the whole, it appears that the usual process of secretion is somewhat as follows: During the period of rest, the cells build up compounds which are preliminary stages of constituents of the secretion, which is afterwards set going by excitation, nervous or chemical. The formation of this material is probably a reversible reaction, so that, after a time, further production ceases, owing to accumulation of products. When the gland is excited to activity, a current of water is set flowing through the cell by some means, probably of an osmotic nature and effected by a combination of increased permeability of the outer end of the cell together with splitting up of some substance into smaller molecules. This current of water washes out into the duct the substances of the secretion already stored in the cell, sometimes after they have been further changed by a process which does not take place until the cells are excited to secretory activity. As the stored substances are lost from the cell, there will be a renewed formation to reestablish equilibrium; so that, if the activity is not too violent, there will be a balance between the amount secreted and its new formation. Continuous secretion will thus be possible without fatigue. It will be seen that, on this view, the increased production in the cell of the substances which give rise afterwards to the actual products contained in the secretion is not to be supposed to be under the control of the nervous system or other excitatory influence, but that it is a spontaneous activity of the cell itself, controlled by chemical equilibrium.

There is no reason to believe that the processes involved in the secretion of milk are different from those as described by Bayliss.

The elaboration of the milk constituents no doubt takes place in the main during the time which elapses between milkings. Vincent (9) cites the work of Lehmann in which a solution of sulphindi-

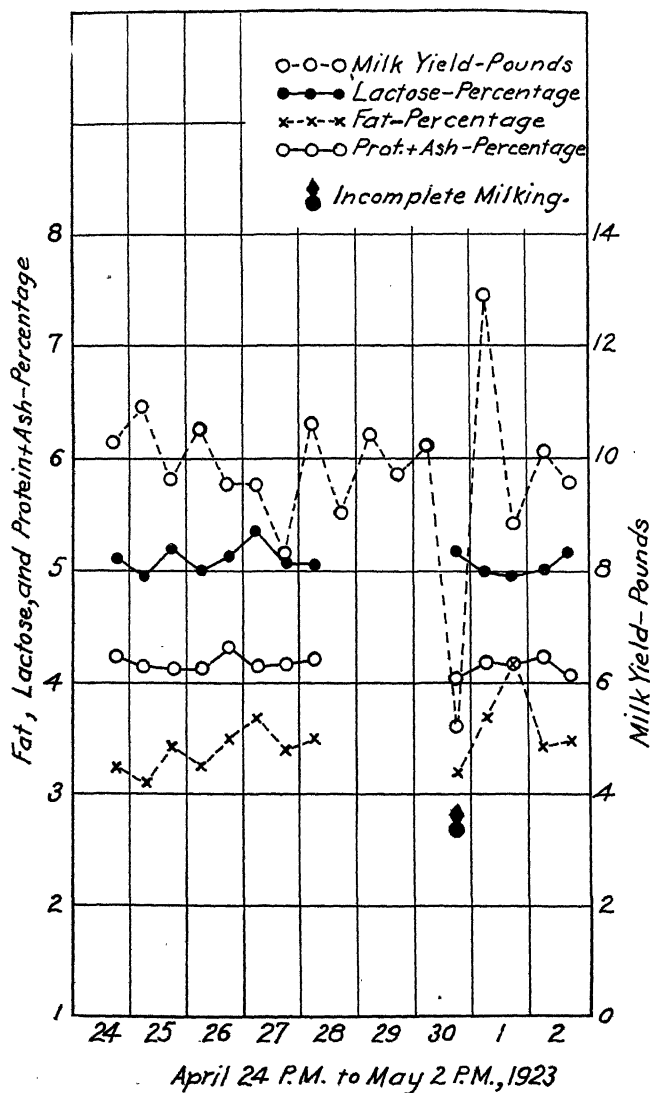


FIG. 5.—Yield of milk and percentage content of lactose, fat, and protein + ash in milk taken at complete milking and at milking where one-half of the average daily evening production was left in the udder

gotate was injected into the vein of a milk goat before milking. In the milking which followed the milk received just before the adders were emptied had a slightly bluish tinge. One hour and a half later the goat was milked again. At this time the milk was

quite blue in color, thus showing that elaboration of the milk constituents took place for the most part between the milkings.

The rate of elaboration of the milk constituents tends to follow the law of mass action, i. e., the rate is inversely proportional to the quantity of the constituents accumulated. Ragsdale et al. (8), working with dairy cows, have shown that, following a milking, each hour's milk production is 95 per cent of the production for the preceding hour. The percentage of total solids in the milk produced at the third hour after milking was 15.6 and at the fourteenth hour after milking it was 12.7. Here, again, we find experimental data which indicate that milk secretion follows the same general law that governs other glandular secretion as described by Bayliss.

Macallum (7) and Garmus (4) have demonstrated that the permeability of a secreting cell changes during that part of secretion which is concerned with the discharge of the secretory products stored in the cell. The membrane at the outer end of the cell next to the lumen becomes permeable, and the stored products, brought about by elaboration, are washed out into the lumen or ducts receiving the secretion. During the discharge of the secretory products there is always a greater concentration on the inside of the secretory cells than on the outside, because of the accumulation of the stored secretory products within the cells. In the case of milk secretion, the process of discharging the secretory products takes place during milking. The stored secretory products (milk constituents) are washed out into the lumen and the solution thus formed is milk. The milk is continually being drawn off during the process of milking, hence there is a continuous flow from a more concentrated solution within the cells to a less concentrated solution in the lumen. This process continues until the udder is milked dry, at which time the secretory cells have completely discharged their contents. With the complete discharge of the secretory products, the concentration within the cells is greatly lowered and at the very end of the process is lower than that of the milk which has been drawn off during milking.⁴ If in case a solution of the same concentration as that of the milk drawn off were returned to the udder at this time it would work its way up into the secretive tissues, as a result of capillary attraction, and there would then be a greater concentration on the outside of the secretory cells than on the inside. The cell membrane immediately after milking (discharge of the secretory products) would still be permeable, and owing to the greater concentration of the solution on the outside of the cells, there would tend to be an absorption (diffusion into the cells) of the solutes present in the solution.

Returning to the results of the experiment (Tables 1 and 2) wherein an isotonic salt solution and an isotonic lactose solution were injected into a quarter of the udder after it was milked dry, it was found that at the milking immediately following, neither the

⁴ This hypothesis as stated does not conflict with the fact that the percentage of fat content of the milk produced at the very end of a milking is much greater than the percentage of fat content of the milk produced at the very beginning of a milking. The most generally accepted explanation of this difference in the percentage of fat contents is given by Kirchner (6). According to this writer the fat globules, especially the larger ones, become mechanically obstructed in the milk ducts of the udder during milking and escape in large quantities in the last milk drawn.

quantity of salt nor the lactose recovered was equivalent to that introduced into the udder in the solutions. Hence, there must have been a partial absorption of both substances by the secretory cells or other cells of the gland. The same must have been true also with respect to the lactose in the milk returned.

The injection of milk or salt and lactose solutions into the udder brought about a marked abnormal variation in the percentage contents of the milk produced during the immediate subsequent milkings, but the injection of distilled water brought about no such abnormal variation. Hence it seems as though this disturbance in the percentage content of the milk is, for the most part, due to the partial absorption of the solutes present in the milk or salt and lactose solutions injected into the udder. It may be possible that this partial absorption of the solutes in the milk or solutions injected produces an abnormal condition in the secretory cells which disturbs their normal functioning. This in turn, of course, would bring about a disturbance in the whole percentage content of the milk produced during the immediate subsequent milkings.

On the other hand, when milk is left in the udder the secretory cells in the secretive tissue never completely discharge their products, and therefore never reach the same condition as when the udder is milked dry. Although there is a film of milk in contact with the cells in this condition, it does not bear the same relation to the cells as it would if the cells had completely discharged their products. The concentration within the cells would still be greater than that of the milk present in the lumen. Hence if there were to be any diffusion of solutes, which no doubt takes place, it would be out of the cells and not into them. When the act of milking ceases the stimulus producing the discharge of the cell products also soon ceases and the whole process is brought to a halt. If there is no absorption of solutes, then there should also be no disturbance in the composition of the milk produced during the subsequent milkings. This reasoning is supported by the fact that there is apparently very little disturbance in the composition of the milk when even one-half of the production is left in the udder, whereas there is a very marked disturbance when only a small fraction of the production is returned after the udder has been milked dry.

SUMMARY OF EXPERIMENTS

A portion of milk was returned to the udder of a cow after it had been milked dry. The effects upon the composition of the milk produced during subsequent milkings were as follows: (1) the percentage of lactose markedly decreased on the first subsequent milking and then gradually returned to its original condition; (2) the percentages of protein and ash increased during the first two or three milkings and then returned to their original condition; (3) the percentage of fat increased very markedly during the first two or three milkings and then became quite variable.

Three hundred cubic centimeters of an isotonic salt solution, 300 c. c. of an isotonic lactose solution, and 300 c. c. of distilled water were, one at a time, injected into the udder after it was milked dry.

The isotonic salt and lactose solutions produced an effect similar to the returning of milk to the udder. The distilled water produced very little disturbance in the composition of the milk.

In order to determine the effect of a partial milking on the composition of the milk subsequently produced, one-fourth, and later one-half, of the average daily evening production was left within the udder. The only percentage that showed any great modification was that of fat content.

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THE EFFECT OF FEEDING CELLULOSE ON THE PULSE RATE OF STEERS¹

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INTRODUCTION

Benedict and Ritzman,³ in their study of undernutrition in steers, observed an almost immediate drop in pulse rate following curtailment of ration, and a very marked increase in pulse rate when their steers were placed on a fattening ration. They concluded that "the pulse rate and metabolism with steers are very closely in agreement," and inferred "that with steers, when there is a change in pulse rate with the same animal, there is a simultaneous change in metabolism which is roughly proportional to this change in the pulse rate." In discussing pulse rates, they stated that "the pulse rate or, more properly speaking, the heart rate, is an index of the relative amount of circulatory activity, and as the blood carries oxygen to the tissues and brings back carbon dioxide to the lungs, the heart rate may be taken as a crude, approximate index of relative metabolic intensity."

In the course of an investigation to determine the effect of crude fiber (paper pulp) on the digestibility of alfalfa by steers, pulse rate data were obtained for the purpose of getting an indication of the metabolic levels of the animals when consuming a ration of alfalfa and when consuming rations of alfalfa and paper pulp in different proportions.⁴ The results were so striking that it seemed desirable to bring them to the attention of other workers in animal nutrition.

EXPERIMENTAL PROCEDURE

In the investigation referred to in the preceding paragraph five "long yearling" steers⁵ were fed for a period of 226 days, and during that time a series of nine digestion trials was conducted. Four of the five steers were used in each of the first two trials and three of them were used in each of the last seven. The digestion trials were 10 days in length and the intervals between them were: (a) 11 days between trials on the same ration, and (b) 18 days between trials on different rations. It was during these nine digestion trials that the pulse rate data herein recorded were obtained.

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³ BENEDICT, F. G., and RITZMAN, E. G. UNDERNUTRITION IN STEERS: ITS RELATION TO METABOLISM, DIGESTION, AND SUBSEQUENT REALIMENTATION. 333 p., illus. Washington, D. C. 1923. (Carnegie Inst. Wash. Pub. 324.)

⁴ The complete data for this investigation are being published elsewhere. See TITUS, H. W. THE MUTUAL INFLUENCE OF THE PROPORTION OF THE SEVERAL NUTRIENTS, IN FEEDS, ON THEIR DIGESTIBILITY: I. CRUDE FIBER—THE DIGESTIBILITY OF RATIONS CONTAINING VARYING AMOUNTS OF ALFALFA AND PAPER PULP. N. Mex. Agr. Expt. Sta. Bul. 153. (In press.)

⁵ Their average initial live weight was 586 pounds and their average final live weight was 774 pounds.

The weight of feed given daily to each steer was 12 pounds ⁶, one half of this being fed at 5 p. m. and the other half at 6.30 p. m. ⁷ One steer received alfalfa and two received mixtures of alfalfa and paper pulp, in different proportions, throughout the investigation; the other two steers received alfalfa part of the time and mixtures of alfalfa and paper pulp the rest of the time (Table 3).

The steers were watered at 3 p. m. and 10 a. m. Pulse rates were taken during the half-hour period preceding each watering; the technic of Benedict and Ritzman was employed. ⁸

COMPOSITION OF THE COMPONENTS OF THE RATIONS, AND OF THE RATIONS

The composition of the dry matter of the alfalfa and paper pulp used in compounding the rations is given in Table 1. The composition (as fed) of the feed used in the nine digestion trials is given in Table 2. Five different rations (four of them definite mixtures of alfalfa and paper pulp) were fed. The percentage of each of the two components in each of the rations was as follows:

	Ration No. 1	Ration No. 2	Ration No. 3	Ration No. 4	Ration No. 5
Alfalfa (per cent).....	100	85	70	55	40
Paper pulp (per cent).....	0	15	30	45	60

The first cutting of alfalfa of very good quality was used throughout the investigation. The paper pulp employed was a high-grade whole rag commercial product. The main constituent of the paper pulp was cellulose which (determined as crude fiber) made up approximately 87 per cent of the dry matter.

TABLE 1.—*Composition of the dry matter of the alfalfa and paper pulp*

ALFALFA

Statistical constants	Ash	Pro- tein	Non- protein	Crude fiber	Ether extract	Nitro- gen- free extract	Total nitro- gen	Pro- tein nitro- gen
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Mean ^a	10.97	14.03	2.12	32.78	1.62	38.48	2.694	2.244
Probable error of mean.....	±.03	±.06	±.02	±.10	±.01	±.08	±.009	±.009
Standard deviation.....	±.19	±.40	±.14	±.70	±.09	±.55	±.064	±.065
Coefficient of variation.....	1.76	2.88	6.74	2.15	5.49	1.42	2.39	2.88

PAPER PULP

Mean ^a	0.94	0.19	Trace.	87.12	0.10	11.64	0.030	0.028
Probable error of mean.....	±.03	±.02	-----	±.25	±.01	±.25	±.002	±.002
Standard deviation.....	±.20	±.09	-----	±1.46	±.04	±1.44	±.009	±.010
Coefficient of variation.....	21.08	46.02	-----	1.67	35.87	12.37	29.11	46.02

^a Computed from the results of 22 separate analyses; the average moisture content of the 22 samples was 8.44 per cent.

^b Computed from the results of 15 separate analyses; the average moisture content of the 15 samples was 5.67 per cent.

^c Except in the case of one steer (No. 44), which was fed 9.5 pounds, 10.5 pounds, and 9.5 pounds per day during the last three digestion trials (Nos. 7, 8, and 9), respectively.

^d The experimental day began at 12 m.

^e BENEDICT, F. G., and RITZMAN, E. G. UNDERNUTRITION IN STEERS: ITS RELATION TO METABOLISM, DIGESTION, AND SUBSEQUENT REALIMENTATION. 333 p., illus. Washington, D. C. 1923. (Carnegie Inst. Wash. Pub. 324.)

TABLE 2.—Composition of the feed used in the nine digestion trials ^a

Ration No.	Digestion trial No.	Moisture	Ash	Protein	Non-protein	Crude fiber	Ether extract	Nitrogen-free extract	Total nitrogen	Protein nitrogen
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1-----	1	8.33	10.25	13.64	1.84	29.68	1.59	34.67	2.573	2.182
2-----	2	8.85	9.80	12.71	2.02	29.84	1.54	35.24	2.464	2.034
3-----	3	8.32	8.64	10.63	1.63	38.06	1.26	31.46	2.047	1.701
4-----	4	9.76	8.17	10.26	1.55	38.52	1.17	30.57	1.971	1.642
5-----	5	8.52	6.99	8.38	1.55	46.61	.92	27.25	1.672	1.341
6-----	6	7.15	7.05	8.55	1.38	47.07	.96	27.84	1.660	1.368
7-----	7	6.57	5.90	6.80	1.05	55.50	.67	23.51	1.310	1.088
8-----	8	5.44	6.01	7.21	1.10	55.86	.67	23.71	1.386	1.152
9-----	9	4.87	4.71	5.06	.79	63.90	.45	20.22	.977	.810

^a As fed.

THE PULSE RATES

The pulse rates are given in Table 3; these are the averages of the afternoon (2.30 p. m.) and morning (9.30 a. m.) pulse rates. The morning pulse rates were almost invariably greater than the afternoon pulse rates; the former averaged 2.8 beats per minute more than the latter. The individual average differences were as follows: Steer No. 41, 3.1; steer No. 42, 5.1; steer, No. 43, 1.6; steer No. 44, 2.2; steer No. 45, 1.9; average, 2.8.

TABLE 3.—The feed and the pulse rates of the steers

Digestion trial No.	Feed given	Average pulse rates ^a				
		Steer No. 41	Steer No. 42	Steer No. 43	Steer No. 44	Steer No. 45
		<i>Beats per minute</i>	<i>Beats per minute</i>	<i>Beats per minute</i>	<i>Beats per minute</i>	<i>Beats per minute</i>
1-----	Ration No. 1-----	59.3	59.3	54.0	63.7	56.7
2-----	do-----	58.0	58.0	52.1	67.9	54.4
3-----	Ration No. 2-----	56.1	56.1	53.8		58.2
4-----	do-----	57.8	57.8	53.8		58.9
5-----	Ration No. 3-----	58.9	58.9	56.1		62.6
6-----	do-----	58.4	58.4	58.2		66.1
7-----	Ration No. 4-----	65.0	65.0	65.3	60.0	
8-----	do-----	62.6	62.6	64.7	61.6	
9-----	Ration No. 5-----	64.1	64.1	66.1	60.9	
(A)-----						
3-----	Alfalfa ⁱ -----	78.1			53.2	
4-----	do-----	70.0			54.0	
5-----	do-----	69.2			52.5	
6-----	do-----	69.1			53.4	
7-----	do-----	64.0				58.4
8-----	do-----	58.7				52.7
9-----	do-----	59.8				54.5

^a The pulse rates given in bold-face figures are the ones around which the discussion centers.^b Pulse rates were not taken. Steer No. 41 was not used in any of the digestion trials.^c This steer had a higher temperature (rectal) than the others during this digestion trial and went "off feed" for several days following this trial.^d This steer had a higher temperature (rectal) than the other steers during this digestion trial and did not eat all the feed offered it.^e A week after the end of digestion trial No. 6 steer No. 45 had an impacted intestine. Steer No. 44 was used in place of steer No. 45 in the last three digestion trials. Steer No. 44 received only 9.5 pounds of feed per day during digestion trial No. 7.^f Received only 10.5 pounds of feed per day.^g Received only 9.5 pounds of feed per day.^h Pulse rates given below the dotted line are for the steers not used in the digestion trials.ⁱ Essentially ration No. 1.

DISCUSSION

The data given in Table 3 show that as rations containing increasing quantities of paper pulp (cellulose) were fed, there was a marked tendency for the pulse rate of the steers to increase. They also show that when (as in the case of steer No. 45) a ration consisting solely of alfalfa was fed in place of one containing paper pulp, the pulse rate dropped immediately; and that when (as in the case of steer No. 44, during the last seven digestion trials)⁹ a ration containing paper pulp was fed in place of one consisting solely of alfalfa, the pulse rate immediately increased, even though the weight of the ration containing paper pulp was less¹⁰ than that containing nothing but alfalfa.

In the case of steer No. 41, which received a ration of alfalfa throughout the investigation, a steady decrease in pulse rate was observed. This animal, unlike the others, was rather nervous and did not become accustomed to its pulse rate being taken until after it had been under observation for several weeks. The drop in pulse rate between digestion trials No. 3 and No. 4 was probably due, at least in part, to the fact that the steer had become accustomed to having its pulse rate taken. A possible explanation of the decrease in pulse rate after the fourth digestion trial is that the fixed weight of alfalfa, which was fed throughout, furnished less and less energy, over and above that required for maintenance, as the weight of the animal increased.

This decrease in the pulse rate of a steer on a fixed ration of alfalfa makes the increase in the pulse rate of the steers receiving rations containing increasing quantities of paper pulp of even greater significance.

If the pulse rate is an index of the relative metabolic intensity, or metabolic level, it would seem as if a given weight of the paper pulp were more efficient than an equal weight of alfalfa in supplying energy. The results of the digestion trials showed that the dry matter of the paper pulp had an apparent digestibility of approximately 77.56 per cent and that the dry matter of the alfalfa had an average apparent digestibility of 59.72 per cent, the latter coefficient of digestibility being to the former as 1 is to 1.3. As will later be shown, this ratio is nearly the same as the ratio of the (calculated) pulse rate which would result from feeding paper pulp alone to that found when alfalfa is fed alone.

In order to secure a numerical measure of the effect produced by paper pulp on the pulse rate, the following equation was used:

$$P = k (A + mB) \text{-----} (1)$$

in which:

P=pulse rate observed when a given ration is fed.

A=amount of alfalfa in the ration (expressed as a fraction of the total ration).

B=amount of paper pulp in the ration (expressed as a fraction of the total ration).

(Note: A+B must equal unity.)

k=pulse rate which would be observed if the ration consisted solely of alfalfa.

m=ratio of the to-be-calculated pulse rate resulting from feeding paper pulp alone to that found when alfalfa is fed alone.

⁹ The pulse rates of steer No. 44 were abnormally high, as were the rectal temperatures during the first two digestion trials, and for that reason can not be compared with the pulse rates obtained during the seven digestion trials which followed. See footnotes c and d of Table 3.

¹⁰ See Table 3, footnotes e, f, and g.

For the sake of convenience, equation (1) may be written in the form:

$$P = kA + kmB \text{-----} (2)$$

or:

$$P = kA + nB \text{-----} (3)$$

in which, $n = km$ (or $m = n/k$), n being the calculated pulse resulting from the feeding of a ration consisting solely of paper pulp.

We may now determine the values of k and n by substituting in equation (3) the values of P , A , and B and solving the resulting equations by the method of least squares.

On treating the pulse rates, given in bold-face figures in Table 3, in the manner outlined above, we get the following values for k , n , and m :

	<i>k</i>	<i>n</i>	<i>m</i>
Steer No. 42.....	57.33	69.52	1.21
Steer No. 43.....	51.51	76.73	1.49
Steer No. 45.....	55.08	83.53	1.52
Average (weighted).....	55.05	73.44	1.33

The values of m for steers Nos. 43 and 45 agree quite well; the value for steer No. 42 is much lower than it is for the other steers. Incidentally, the difference between the morning and the afternoon pulse rate of steer No. 42 was greater than that of any of the other steers and, in general, the pulse rate of this steer showed the greatest fluctuations. This may account for the low value of m obtained with steer No. 42. In view of the fact that the conditions under which the pulse rates were taken were not rigidly controlled, it seemed best to treat the pulse rates of these three steers as though they were for one animal, and in this way secure an average value of m . This average value of m was found to be 1.33, which is very nearly the same as the value of the ratio of the coefficient of apparent digestibility of alfalfa to that of paper pulp. The agreement between these two ratios seems to indicate that the digestible portion of paper pulp is at least equivalent to the digestible portion of alfalfa as a source of energy.

Although Benedict and Ritzman¹¹ considered the pulse rate as a crude, approximate index of the metabolic intensity, it may be that if a highly refined technic were worked out for securing an average pulse rate over an extended period of time, this pulse rate would be a rather accurate index of the metabolic intensity and of great worth in comparing the nutritive values of feeding stuffs.

CONCLUSION

Cellulose (in the form of paper pulp), when substituted for an equal weight of alfalfa, in a ration consisting solely of alfalfa, tends to cause an increase in pulse rate, which is proportional to the amount substituted.

¹¹ BENEDICT, F. G., and RITZMAN, E. G. UNDERNUTRITION IN STEERS: ITS RELATION TO METABOLISM, DIGESTION, AND SUBSEQUENT REALIMENTATION. 333 p., illus. Washington, D. C. 1923. (Carnegie Inst. Wash. Pub. 324.)

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BOTRYTIS NECK ROTS OF ONIONS¹

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INTRODUCTION

The serious nature of losses due to *Botrytis* infection of onion bulbs first came to the writer's attention in the late autumn of 1915. During the subsequent winter the damage from this malady in mid-western onion-growing districts was of such magnitude as to leave no doubt as to its importance. Investigations were begun in 1917 upon the nature and causes of the disease and possible remedial measures. About the time this work was started, Munn (20)² published his report in which the cause of neck rot of onion was attributed to *Botrytis allii* Munn. His findings, in so far as they relate to the cause of the disease, have in the main been confirmed. In addition it has been found that a second species, *B. byssoidea* Walker is the more common cause of neck rot in Wisconsin and Illinois sections, and that a third species, *B. squamosa* Walker also causes a neck-rot decay (34).

It is the purpose of the present paper to review briefly the history of neck rot as so far treated in the literature, and to report studies upon the three forms of neck rot which are now known to occur. Since each of these three forms produces distinct symptoms and is caused by a distinct species of *Botrytis*, the three maladies must in any critical consideration be regarded as separate diseases. On the other hand, their similarity in so many respects is justification for referring to them by a group name which will indicate that they are similar diseases caused by closely related organisms. There are several reasons for allowing the term "onion neck rot" to apply to the group. It is an accurate and adequate common term already in good usage; it was probably applied to one or all of the three diseases before any clear differentiation was made; and all three parasites invade the onion bulb chiefly through the neck tissues.

For differentiating between the three types of neck rot it has been suggested that descriptive adjectives be used (34). For the form already described by Munn (20) as due to *Botrytis allii*, the term "gray-mold neck rot" is used because of the prompt and abundant production of gray conidial masses upon the decaying host tissue. For the second form, due to *B. byssoidea*, the name "mycelial neck rot" is suggested because of the predominance of mycelium and the

¹ Received for publication July 13, 1926; issued December, 1926.

² Reference is made by number (italic) to "Literature cited," p. 926.

scarcity of conidia on the decayed host tissue, as compared with the previous form. For the third form, due to *B. squamosa*, the name "small sclerotial neck rot" is suggested because the appearance of small sclerotia upon affected scales (i. e., small as compared with those of the other two forms) is an early and fairly constant sign of the disease.

HISTORY AND DISTRIBUTION OF ONION NECK-ROT DISEASES

The first description of onion neck rot was apparently that by Sorauer in Germany in 1876 (25). He again described it in the second edition of his textbook, in 1886 (26, p. 294-297). The symptoms which he has recorded correspond closely with those later given by Munn (20). He considered the form of *Botrytis* causing the disease to be *Botrytis cana* (Pers.) Fr. and the sclerotia probably to be identical with those which had been previously described as *Sclerotium cepae* Berk. and Br. He inclined to the view that the latter would give rise to an ascigerous stage identical with *Sclerotinia fuckeliana* but did not adduce any evidence to substantiate this view. It is likely, considering the size of conidia which he reports, that the form he had under observation was identical with *B. allii* Munn. He was able to reproduce the disease by artificial inoculation of healthy bulbs with conidia, but noted that the success of such inoculations depended on the presence of humid conditions. The severity of the disease in the field was dependent to a marked degree upon the amount of humid weather which prevailed about harvest time, a factor which later workers have likewise found to be of great importance. He was unable to produce infection upon healthy, vigorous, green onion leaves. He noted the conspicuous varietal differences in susceptibility, which have been recorded many times since, namely, that the white type of onion is very susceptible, whereas the yellow and red types are much less seriously affected.

Frank (8) described the disease, also from Germany, in his textbook in 1880. He added little to the facts given by Sorauer except that he claimed to have produced infection upon the green leaves of onion by means of artificial inoculation. He considered the causal organism to be identical with *Botrytis cinerea* Fr. Smith (24) in 1900 discussed the onion neck-rot disease which he found at Munich, Germany, and pointed out that the organism was distinct from *B. cinerea* both with respect to the shape of conidia and with regard to the low, creeping growth of the fungus as it sporulated upon decaying onion tissue. He was most likely working with *B. allii*.

A neck-rot disease was first reported from England by Massee (18) in 1894. Though he attributed the cause to *Sclerotinia bulborum* Wakker, experimental proof of the causal relations is lacking. An illustration showing the disease, in a later publication (19), indicates that he was dealing with either *Botrytis allii* or *B. byssoides*. Cotton and Owen (7) later reported the occurrence of *B. allii* in England.

In 1903 Voglino (29) distinguished the *Botrytis* disease of onion found in Italy from that caused by *Sclerotium cepivorum* Berk. and referred the neck-rot fungus to *S. ambiguum* Duby.

Hanzawa (10) in 1914 reported a *Botrytis* disease of onion bulbs in Japan. He gave the size of conidia as 8.4 to 16.8 by 6.3 to 10.5 μ and considered that it was probably identical with *Botrytis cinerea*. These measurements correspond most closely with those of *B. byssoidea*, but it is impossible to determine with certainty from his meager description whether the form he studied was *B. allii* or *B. byssoidea*.

Halsted (9), the first to record the disease in America, gave a brief report of its occurrence in New Jersey in 1890, and referred to the causal organism as *Botrytis parasitica* Cav. Clinton (4, 5) reported serious epiphytotics of neck rot upon white onions in Connecticut in 1902 and 1903. He reported that the fungus caused yellowish spots on the leaves of the onion plant in July and also blasted the flowers of the seed plant. He pointed out the importance of humid weather in bringing about heavy infection and attributed the absence of the disease in 1904 largely to the dry weather which prevailed during July and August of that year.

Selby (23), writing in 1910, considered this to be the most serious disease of white onions in Ohio. He erroneously identified the causal agent as *Sclerotium cepivorum*. This fungus, described originally by Berkeley (1), produces minute sclerotia, while the diseased specimens illustrated by Selby are characteristic of those produced by *Botrytis allii* and by *B. byssoidea*. Humbert (13), writing on the same disease from Ohio, also referred to the causal organism as *S. cepivorum*, but that this interpretation is erroneous is obvious from his description of conidia and his illustration of a diseased onion bulb, which is similar to that published by Selby.

A neck-rot disease of onion was destructive to onions in Oregon in 1912, according to Jackson (14), and from his description it is apparent that *Botrytis allii* was the chief causal agent in that case. Munn (20) found neck rot repeatedly in New York and Michigan from 1913 to 1916, and described the disease as caused by *B. allii*. The data accumulated by the Plant Disease Survey (3, 6, 11, 15, 28) from 1921 to 1925 are sufficient to show that onion neck rot is widespread in this country. Although more consistent losses are perhaps sustained in the North, most onion-growing areas suffer from time to time, while the damage in a given locality varies widely with the season.

In many of the references cited above it is hardly possible to distinguish between the three types of neck rot. In practically all of the instances noted it is quite evident that the causal organism was either *Botrytis allii* or *B. byssoidea*. *B. squamosa* seems heretofore to have attracted little attention. *B. allii* has been isolated by the writer from bulbs grown in California, midwestern States, Pennsylvania, and Connecticut, and Munn (20) obtained it from Michigan and New York bulbs. Moreover a culture isolated from onions in France and forwarded to the writer by the Centralstelle für Pilzkulturen at Baarn, Holland, under the name of *B. cana* was found to be identical with *B. allii*. The mycelial neck-rot form, *B. byssoidea*, has been isolated from bulbs grown in midwestern States and in Connecticut, and was found by the writer on onions in the market at Paris, France, in 1922. It would appear, therefore, to be quite as widespread in its occurrence as *B. allii*. The small sclerotial

form, *B. squamosa*, has so far been isolated by the writer only from onions grown in the Middle West, but no extensive search has been made for it elsewhere.

BOTRYTIS AND SCLEROTIUM FORMS PREVIOUSLY DESCRIBED ON ALLIUM

The Botrytis and Sclerotium forms upon species of *Allium* which have been described in literature present a very confusing situation to the reader. Many of these are the result of collections made on dying or dead parts and may or may not have a connection with the neck-rot forms. One of the first sclerotial forms on *Allium cepa* to be described in Europe was *Sclerotium cepivorum* Berkeley (1). A recent survey has shown this to be widespread in Europe and to occur in America (31). There should be no cause for confusing it with the Botrytis forms on onion for it lacks conidia and has much smaller sclerotia, but nevertheless it has been confused with these forms by Sorauer (26), Bruck (2), Selby (23), and Humbert (13). Massee (18) refers to the form associated with neck rot as identical with *Sclerotinia bulborum* Wakker on hyacinths, but there seems to be no justification for his statement. Voglino's reference (29) of the Botrytis found on *Allium cepa* to *Sclerotium ambiguum* Duby seems unwarranted. The identity of the neck-rot Botrytis with *Botrytis cinerea* has been suggested by Frank (8) and Hanzawa (10), but the differentiation between the latter and the onion form from Germany with which he worked was pointed out by Smith (24), and the distinction between *B. cinerea* and *B. allii* was later made by Munn (20). There is no ground for considering either of the Botrytis forms on onion identical with *B. cana*, as suggested by Sorauer (26), with *B. aelada* Fres. (21, v. 4, p. 131), with *B. fulva* Link (16, Abt. 8, p. 280-281), or with *B. vulgaris* Fr. var. *interrupta* Fr. (21, v. 4, p. 129) the last three of which have been found on dead scales or stems of *Allium*. *B. parasitica* Cav., reported on *Allium ursinum* (16, Abt. 8, p. 292), and referred to by Halsted (9) as the cause of onion neck rot, has been studied recently by Hopkins (12) and is undoubtedly distinct from all the onion forms.

Saccardo (21) describes several species of *Sclerotium* which have been found on living or dead parts of various species of *Allium*. It is not likely that any of these forms are identical with either of the sclerotial forms associated with onion neck rot. *Sclerotium ambiguum* Duby, *S. inconspicuum* Lib., *S. pulveraceum* Dur. and Mont., and *S. sepicorum* Berk. (21, v. 14, p. 1150-1151) are all described as having very small sclerotia, smaller than those of *Botrytis squamosa*. A form producing thin, ovate-oblong, minute sclerotia, described as occurring on *Allium vineale*, suggests some similarity to *B. squamosa*, but this form was ascribed to *S. tulipae* Lib. var. *hyacinthi* Guep. (21, v. 14, p. 1172), which has been described fully by Hopkins (12) and presents no close similarity to the onion neck-rot forms. *S. brassicae* Pers. (21, v. 14, p. 1164-1165), and *S. durum* Pers. (16, Abt. 9, p. 674-675) are described as producing very large sclerotia. The former, found on *Allium victorialis*, yielded no Botrytis form, while the latter was said to produce a Botrytis stage identical with *B. cinerea*.

An undescribed species of yellow-spored form of *Aspergillus* was isolated from Italian-grown garlic by E. D. Eddy in 1919. This

species was found by the writer to be pathogenic upon onion bulbs as well as upon garlic. It produces in the decayed bulb tissue dark brown to black, slightly elongate sclerotia 1 to 2 mm. in length, which might be confused with other forms. The yellow color of the spores and the brown cast of the sclerotia are the chief characters by which it is readily distinguished from the above-mentioned sclerotial forms on onion. The causal organism has been recently described as *Aspergillus alliaceus* Thom and Church (27).

Sclerotium rolfsii is sometimes found on bulbs of garlic, but as yet no occurrence on onion bulbs has come to the writer's attention. The sclerotia are readily distinguished from those of the above form by their decidedly lighter brown color.

Sawada (22, p. 206-209) has described a disease of *Allium cepa* and *Allium fistulosum* which occurs in Japan and Formosa. This is attributed to a newly described species, *Sclerotinia allii* Sawada. The malady is one which attacks the leaves and stems of the growing plant, however, and is not reported as a storage decay of bulbs. Sawada states that *Sclerotinia libertiana* Fuckl. (*Sclerotinia sclerotiorum* (Lib.) Masee) also attacks onion plants in Japan, but so far as the writer is aware this fungus has not been authentically described elsewhere as an onion pathogene.

GRAY-MOLD NECK ROT

SYMPTOMS

Gray-mold neck rot is found most commonly upon the bulbs after harvest, infection taking place most readily through the neck tissues. The first sign of the disease is the softening of the affected scale tissue, which takes on a sunken, cooked appearance characteristic of the advancing zone of the diseased areas. The margin between the diseased and healthy tissue is quite definite, and since the effect upon the host cells is produced somewhat in advance of the hyphae, usually little evidence of the fungus is visible there. As the mycelium multiplies in the older diseased area the affected tissue becomes grayish in color, and later a dense grayish mycelial mat often develops upon the surface of the scale. Under average conditions, conidial production is prompt. A dense layer of gray mold consisting of comparatively short conidiophores and myriads of conidia is produced on the outer diseased scales. The early production of spores, even under average room conditions, is characteristic of this form, whereas the other two forms are more tardy in sporulating.

The disease progresses most rapidly down the scales which have been originally infected in the neck, while the spread from one scale to another proceeds somewhat more slowly. In a cross section of a partially rotted bulb, at the advance margin of the disease, the parenchyma has a distinctly water-soaked appearance. A few millimeters above this point a grayish tinge and slight shrinkage in the decaying tissue become evident, while still farther up the shrinkage of the scales and the development of mycelium increase. On the outer scales conidial production is pronounced. Sclerotia occur in connection with the older decayed tissue, first as whitish mycelial masses, which become darker with age until they assume the appearance of hard, black, rounded, kernel-like bodies, spherical, oblong, or irregular, and

varying from 1 to 3 mm. or more in length. They form usually on the outer surface of the scale or are slightly embedded in the diseased host tissue.

Occasionally infection takes place through the bases of onion scales or through wounds. The results—the progress of the disease and the appearance of the tissue—in such cases are quite similar to those in cases of neck infection. The older decayed bulb presents the appearance of a “mummy.” While the host tissue in the beginning of its decay is somewhat watery, it desiccates rather promptly and does not give the appearance of being affected by common bacterial soft rot. The moisture which is released is often sufficient, however, to stimulate growth of the central bud, and the premature sprouting of affected bulbs is a common sign of the disease. Little or no offensive odor is occasioned by neck-rot decay. Commonly, however, soft-rot bacteria follow close upon *Botrytis allii*, in which case a more watery decay and more odor result. Certain phases of the disease are illustrated in Figure 1.

Botrytis allii, being a facultative saprophyte, develops commonly upon dead onion parts and other refuse in the field. Munn (20) reports finding it also upon the roots, leaves, and flower parts of the onion plant. It would appear from his experiments that the organism may act as a parasite upon the aerial parts of the growing plant. The writer has never found any of the three *Botrytis* forms attacking the actively growing parts of the plant. The older dying leaves and the tips of leaves turning yellow from other cause often show *Botrytis* conidial production during moist weather, but the actual initial damage caused by *Botrytis* is not ordinarily noticeable under mid-western conditions.

THE CAUSAL ORGANISM (*BOTRYTIS ALLII*)

The septate, branching mycelium of *Botrytis allii* has little in the way of distinctive characters. It is hyaline for the most part, sometimes taking on a slight tinge of color with age. The aerial hypae en masse have a smoky-gray cast, but only in the conidiophores do the walls take on a deep-brown color.

Appressoria, or holdfasts, are commonly formed by repeated branching of hyphae which comes into contact with the host surface or with the glass containers of pure cultures. The mycelium in these bodies for the most part contains denser cytoplasm than is found elsewhere, and the walls commonly are darkened in color, giving often a very dark macroscopic appearance to the holdfasts.

Sclerotia are commonly formed in or upon the decayed host tissue. The conditions which promote the formation of these resting bodies have not been worked out. It is not uncommon for an onion bulb to suffer complete decay without the production of sclerotia, but usually abundant sporulation takes place. Newly formed sclerotia appear as white, dense, velvety masses of mycelium, which soon darken somewhat. As development continues the surface assumes a waxy appearance, light brown at first, gradually becoming darker, until a hard, black, kernellike mass results. The mature sclerotia are variously shaped; they are rounded on the upper surface and are either flat or concavely depressed on the lower side when attached to the outer surface of the onion scale. They vary

in size from 1 mm. or less to 4 or 5 mm., but most of them measure from 2 to 4 mm. It is not uncommon, however, to find many sclerotia aggregated into a crusty mass of considerable size. In

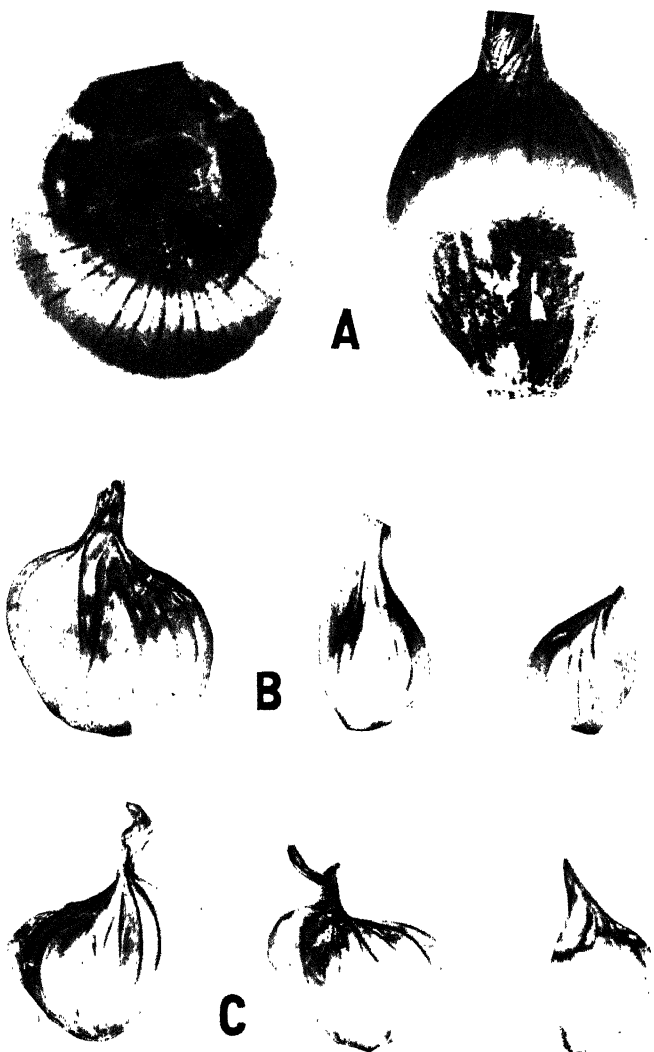


FIG. 1.—A, gray-mold neck rot (*Botrytis allii*). Natural infection. At left, Crystal Wax variety showing neck infection; at right, White Globe variety showing infection through base of bulb. Note characteristic gray mold on the decayed tissue. B, Crystal Wax bulbs inoculated with *Botrytis allii* through needle wounds, placed in moist chambers, and incubated (left to right) at 18°, 23°, and 26° C., respectively, for eight days. C, Crystal Wax bulbs inoculated with *Botrytis byssoides* but handled otherwise like those in B. Note in B and C that the decay was most rapid at 18° C., somewhat less rapid at 23°, and very meager at 26°

cross section they exhibit a pseudoparenchymatous structure consisting of closely packed mycelial threads. The several outer layers consist of dark-walled cells which give the external black color to

the bodies; the remaining interior is made up of hyaline cells. Under suitable conditions the sclerotia germinate either by sending out hyaline mycelial branches or by the direct production of conidiophores upon their surfaces.

The conidiophores are produced directly from the free mycelium in the tissues or from sclerotia. Branches are sent up approximately at right angles to the mycelial thread. (Fig. 6, A.) These are hyaline at first and remain so in the region of the growing tip, but the older portions of the conidiophore walls become gradually darker until they are a deep brown. They vary from $6\ \mu$ to $20\ \mu$ or more in diameter, are septate and occasionally branched, and usually attain a fairly uniform height above the surface of the host. With their profuse production of spores, they give a low, dense, gray-mold appearance, often distinguishable from the higher and less-compact habit of *Botrytis byssoides*. The growing tip of the conidiophores preliminary to spore production sends out several side branches from the main stem and these rebranch once or twice. (Fig. 6, B.) The ends of these branches become rather swollen, and from these rounded tips the sterigmata which later produce the conidia are sent out in large numbers. (Fig. 6, C, D.) These sterigmata, after attaining a length of $1\ \mu$ to $2\ \mu$, swell at their tips to form the spores. This takes place almost simultaneously on all the sporiferous tips. (Fig. 6, E.) While the spores are enlarging they are not readily detached from the conidiophore, but when they are mature they disperse readily when placed in liquid or when exposed to air currents. Cleavage of the spore usually takes place at the top of the sterigma, and the sterigma is not usually attached to the spore after dispersal. About the time of spore maturation, cross walls are laid down, first just behind the sporulating tips and finally in close proximity to the main stalk of the conidiophore. Degeneration begins above these septa, starting with the sterigmata and continuing with the sporiferous branches. (Fig. 6, F.)

The main branch of the conidiophore proliferates by extending upward (fig. 6, G), and in due time it again branches and repeats the process of sporulation. Each point of sporulation on the main stalk is marked by slight scars or knobs on the conidiophore where the septa were laid down to cut off the degenerating sporulating branches. (Fig. 6, H.) By this time the walls have usually become dark colored. The conidiophore may thus sporulate and proliferate several times during the course of its development, and the region of sporulation is marked each time by the group of scars where the branches were cut off, while the conidia remain grouped around these points if no liquid chances to come in contact with them or if they are not blown away by air currents. With age the conidiophore becomes flattened and twisted. Thus in nature it is not uncommon to find conidiophores 1 or 2 mm. in length with groups of spores adhering to them at regular intervals. Occasionally one of the sporulating branches does not degenerate but also proliferates and continues to sporulate, thus giving rise to the occasional branch found with the conidiophores of *Botrytis allii*.

In describing this phase of the morphology of the organism, Munn (20) does not mention the branching of the conidiophore previous to sporulation, and in describing and figuring the scars upon

the main stalk he intimates that the conidia are produced directly upon them. It is not always easy to demonstrate the exact origin of the conidia, chiefly because the mature conidia disperse as soon as placed in water mount, but after repeated examination of the sporulating tips before the spores are mature and thus not readily detached, there is no reason to doubt that they are always formed in the manner just described.

The conidia are oblong to elliptical in shape, hyaline, and continuous. (Fig. 6, I.) They vary in size over a considerable range (6 to 16 by 4 to 8 μ), but measurement of a considerable number shows that a majority fall within the range of 7 to 11 by 5 to 6 μ . The frequency distribution of length and width for a number of strains growing on onion tissue and on potato agar, as given in Table 1, show a reasonable degree of constancy in range of size. By comparison with measurements of several strains of *Botrytis byssoides* and of *B. squamosa* given in the same table, it is evident that *B. allii* falls in a class the average measurements of which are somewhat smaller than those of the other two species which affect onion bulbs. Germination takes place under favorable conditions in 12 to 14 hours by the production usually of a single germ tube, occasionally of two, and almost always without septation in the spore during germination. Microconidia are not common; they are globose, about 3 μ in diameter, and are borne on short hyaline conidiophores.

TABLE 1.—Conidial measurements from a number of strains of *Botrytis allii*, *B. byssoides*, and *B. squamosa* ^a

Species	Strain No.	Source	Substrate	Percentage of spores falling within the length (in microns) of groups indicated																			
				6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
<i>Botrytis allii</i> ...	64	Louisiana	Potato agar	10	32	34	16	6	2														
			Onion		12	36	44	8															
	65	(v)	Potato agar	10	26	28	24	6	4			2											
			Onion	2	16	36	36	10															
	76	Illinois	Potato agar	2	10	14	14	22	10	14	4	4	2	4									
			Onion			4	38	40	10	4	2	2											
	77	do	Potato agar	8	14	22	30	16	2	4	4												
			do	12	22	24	28	10	2	2													
	83	Ohio	Onion		18	56	20	6															
		do	Potato agar	4	36	30	16	12	2														
<i>Botrytis byssoides</i>	88	Pennsylvania	do	2	20	24	26	20	4	2	2												
	96	Illinois	Onion			32	40	20	4	4													
	100	California	do	12	36	44	8																
	42	Wisconsin	do		2	2	4	2	12	12	30	22	2	8	2	2							
	70	Illinois	do					2	6	10	28	28	14	4	2	6							
	80	Wisconsin	do					4	12	26	14	20	12	2	4	2	2	2					
	89	Illinois	Potato agar					2	30	22	20	20	6										
			Onion		6	8	50	30	6														
	90-1	do	Potato agar					6	30	44	16	2	0	0	2								
	90-7	do	do					6	6	20	34	16	12	6									
<i>Botrytis squamosa</i>	90-7	do	do					2	10	16	26	16	14	6	8	2							
	98	Wisconsin	Onion leaf							20	52	12	8	8									
	101	do	do							4	36	16	44										
	114	France	Onion		2	5	17	45	23	7	1												
	94	Illinois	do							4	32	20	28	12		4							
	27	Wisconsin	Potato agar									2	6	16	20	18	12	20	6				
	30	do	do									2		8	6	8	22	18	18	12	6		
134	do	do										3	4	15	15	17	17	11	7	9	1		

^a In most cases 100 spores from each strain were measured.
^b Culture No. 65 was furnished by M. T. Munn, New York State Agricultural Experiment Station.

TABLE 1.—*Conidial measurements from a number of strains of Botrytis allii, B. byssoides, and B. squamosa*—Continued

Species	Strain No.	Source	Substrate	Percentage of spores falling within the width (in microns) of groups indicated														
				4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Botrytis allii</i>	61	Louisiana	{ Potato agar	58	40	2												
			{ Onion	18	66	16												
	65	(b)	{ Potato agar	2	88	10												
			{ Onion	6	42	42	10											
	76	Illinois	{ Potato agar	4	68	26	2											
			{ Onion	18	60	20	2											
	77	do	Potato agar	6	80	14												
	83	Ohio	{ do	64	36													
			{ Onion	38	60	2												
	86	do	Potato agar	4	58	36	2											
<i>Botrytis byssoides</i>	88	Pennsylvania	do	62	38													
	96	Illinois	Onion	24	68	8												
	100	California	do	36	48	16												
	42	Wisconsin	do	2		26	56	14	2									
	70	Illinois	do		6	24	28	32	10									
	80	Wisconsin	do	4	5	34	36	18	2									
	89	Illinois	{ Potato agar		6	28	32	26	8									
			{ Onion	2	28	62	8											
	90-1	do	Potato agar			68	30	2										
	90-5	do	do			24	60	14	2									
<i>Botrytis squamosa</i>	90-7	do	do			6	38	26	12	4	4							
	98	Wisconsin	Onion leaf			8	60	28	4									
	101	do	do	24	28	48												
	114	France	Onion		17	74	9											
	94	Illinois	do		20	44	32	4										
	27	Wisconsin	Potato agar						2		12	38	24	22	2			
	30	do	do						2		4	22	36	24	10	2		
<i>Botrytis squamosa</i>	134	do	do						3	8	30	29	9	10	7	3		1

° Culture No. 65 was furnished by M. T. Munn, New York State Agricultural Experiment Station.

PATHOGENICITY

Munn (20) has already given conclusive proof of the pathogenicity of *Botrytis allii* upon onion bulbs. Many experiments have been performed by the writer in which the conidia or mycelium have been injected into wounds in the succulent scales. In general, when too rapid desiccation of the wounds was prevented and the bulbs were kept at any temperature between 5° and 20° C. positive infection resulted uniformly. The most satisfactory method of obtaining infection has been to inject conidia into needle wounds, placing bulbs in oiled paper bags and incubating them at 15° to 20°. Infection of bulbs by spraying spores on the unwounded surfaces of dry or succulent bulb scales was found by Munn (20) to be difficult to attain. The writer has likewise failed to produce infection by this means. It is evident, however, that the fungus may penetrate the unbroken cuticle of succulent scales. This was demonstrated by carefully inoculating the outer succulent scale of a bulb in the usual manner. After the resulting lesion had advanced to a certain point the outer scale was removed, and invasion of the adjacent underlying scale was found to have occurred. This experiment was repeated several times with uniform results. From this fact it is evident that when natural infection of a single scale takes place at the neck or through wounds at other points on the bulb the fungus is not necessarily confined to the scale originally infected. It is true that the fungus does spread more rapidly through the tissue of the first scale infected, but

advance into adjoining scales is not precluded. However, initial infection through the unwounded outer scales apparently does not occur, or in any case is exceptional. When inoculation is made in the succulent tissue, the fungus decays the bulbs of colored and white varieties equally well. The nature of resistance of colored bulbs is discussed later in this paper.

When the onion plant is mature, infection may occur through the unwounded neck tissues, most of which by that time are dead or senescent. This has been shown by artificially inoculating bulbs with and without tops. For further discussion of these experiments the reader is referred to a subsequent section on "Relation of stage of maturity of host to infection of bulbs."

Munn (20) produced infection repeatedly by applying spores to the green leaves of plants in a humid atmosphere. Two experiments were performed by the writer in which spores were applied to leaves of vigorously growing plants which were then placed in a moist chamber at about 18° C. for 48 hours. Fine droplets of water covered the plants during this period, and conditions should have been ideal for infection. The plants were removed from the chamber to a greenhouse in which the temperature ranged from 15° to 20°. No infection became evident in either experiment. Several experiments were then performed in which the plants were left in the moist chamber for longer periods. After the first week the infection of some of the leaves was evidenced by the softening of the affected tissue, and this softening was followed by the characteristic sporulation of the organism on the decayed areas. Under these conditions, results quite similar to those of Munn were obtained. It seems to be necessary, however, to keep the plants in a very humid environment for fairly long periods in order to produce infection. When affected plants were removed to ordinary greenhouse environment, the progress of the fungus was almost, if not entirely, checked. The writer is inclined to consider the disease of the aerial parts other than the bulb caused by this fungus as a very mild type of parasitism, which takes place only under extreme and abnormal conditions. This evidence, together with field observations, would lead one to conclude that *Botrytis allii* is of strictly limited importance as a parasite of the aerial portions of the plant. Under extremely humid conditions it might cause slight damage, but how much of the action was truly parasitic and how much was mere subsistence upon senescent or dead tissue would remain an open question. Its occurrence on senescent or dead tissues is no doubt common, and its development upon dead leaves and dead tips of leaves during the growing season of the host is undoubtedly an important part of the life cycle of the organism. That the occurrence of *Botrytis* apparently damaged onion leaves has been reported in a few instances (4, 5, 11, 17, 20).

HOST RANGE

By artificial inoculation, bulbs of white multiplier onion (*Allium cepa* var. *bulbellifera*) and white shallot (*A. ascalonicum*) were found to be quite as susceptible to gray-mold neck rot as those of common onion. Garlic bulbs (*A. sativum*) were also readily infected, but the progress of the disease was much slower on these than on either of the hosts just mentioned.

MYCELIAL NECK ROT

SYMPTOMS

In the early stages of its development the mycelial neck rot is not readily distinguishable from the gray-mold neck rot. As in the latter, the usual avenue of infection is through the neck tissues, but occasionally cases are found where the invasion takes place by way of the stem plate or through wounds at other points upon the onion scales. The same water-soaked, sunken appearance typifies the newly infected tissue, and the advancing margins of the diseased area continue to show this symptom. The line of demarcation between healthy and diseased tissue is also usually well defined, and as in the case of *Botrytis allii*, the mycelial neck-rot organism initiates the breakdown of the host tissue somewhat in advance of the hyphae. In the older decayed parts the scales become shrunk, and grayish superficial mycelium develops in a manner not unlike that of *B. allii*. There is in the main, however, a greater quantity of superficial mycelium formed in the case of mycelial neck rot than would be the case with gray-mold neck rot under like environment. Recently inoculated bulbs of the two forms placed in moist chambers respond quite differently. In the case of gray-mold neck rot the organism sporulates promptly, with little additional superficial mycelium, while *B. byssoides* usually produces a profuse quantity of mycelium, white at first but later turning gray with age. The most striking point of difference between the two forms is that the mycelial neck-rot organism sporulates very sparsely in these early stages, and it is often difficult to bring about conidial production on newly decaying bulbs even when the latter are placed in the moist chamber. As the mycelial neck rot progresses sclerotia commonly develop in the older decayed tissue. They resemble very closely those of *B. allii* in all respects. Sporulation occurs to some degree under sufficiently moist conditions on the older decayed tissue. The sporulating mass is light-gray at first, gradually becoming darker with age, the darker shade being due in the main to the wall coloration in the older portions of the conidiophores. As a rule the sporulating mass is distinguishable from that of *B. allii* because of the fact that the conidiophores of the latter are usually somewhat shorter, less branched, and more prolific, giving the appearance of a low dense mass of conidia. In the case of *B. byssoides*, on the other hand, the conidiophores assume somewhat greater length, are more branched, and sporulate less profusely, giving in the end the appearance of a more fluffy development of aerial mycelium with a less dense conidial mass.

It will be seen from this description that points of macroscopic difference between the symptoms of the two diseases are not sufficiently marked to afford even the trained casual observer a ready means of distinction. The outstanding macroscopic distinguishing characters which one may use with reasonable accuracy after some experience in observing the two diseases are (1) the comparative lack of sporulation in mycelial neck rot and the abundance of sporulation in gray-mold neck rot, and (2) the fact that when sporulation does occur in mycelial neck rot, the ranker growth of conidiophores is usually noticeably different from the low dense growth of the sporulating hyphae of the gray-mold neck rot. Distinction is made the more difficult by the fact that at times the two forms may be found

together upon the same bulb. Very often one who is working constantly with the two diseases finds it necessary to resort to artificial culturing of the organisms to make diagnosis certain. Growing the organisms upon potato-dextrose agar is a ready means of distinguishing between them. (See fig. 5.) *Botrytis allii* will invariably sporulate abundantly with a few days, while the mycelial neck-rot organism produces rank-growing, fluffy, white aerial mycelium, with seldom, if ever, any evidence of sporulation. Certain aspects of the disease are shown in Figures 2 and 3.

THE CAUSAL ORGANISM (BOTRYTIS BYSSOIDEA)

The mycelium of *Botrytis byssoides* has no characters by which it may be readily distinguished from *B. allii*. The appressoria or holdfasts are also very similar in the two species. Sclerotia are formed in the same manner and are much alike as to size, shape, and color.

The conidiophores of *Botrytis byssoides*, like those of *B. allii*, originate either from the free mycelium or from the sclerotia, and their development and the method of spore production in the two forms is of the same general type. A few points of difference in detail are to be noted. Conidiophores are produced much less commonly on the decayed host tissue in *B. byssoides*, and they are seldom found on artificial media. When produced upon decaying onion scales they are noticeably more scattered than the conidiophores of *B. allii*; permanent branches are formed a little more commonly; and the production of spores is not so profuse. The conidiophores are hyaline at the growing tip, and the walls turn brown promptly as they grow older. To the naked eye the fungus as it fruits upon onion tissue presents a less crowded, more cottony, and somewhat more elevated appearance than the low dense gray-mold development of *B. allii*. The fertile tips of the conidiophores branch and produce spores in the same manner as described in connection with *B. allii*. (Fig. 6, M.) The sporiferous side branches usually degenerate as far back as the septum, which is laid down close to the main trunk of the conidiophore (fig. 6, N); occasionally one persists and continues to grow out as a permanent fertile branch. In this form, as in *B. allii*, the branches, whether permanent or not, show a marked constriction at the point of juncture with the main trunk when they have reached their maximum diameter, and in the case of the permanent branch this constriction remains. As in the other forms, it is common for the conidia to adhere to the sides of the main trunk of the conidiophore until they are removed by water or air currents. The scars left by the degenerated fertile branches are quite similar to those of *B. allii*.

The conidia of *Botrytis byssoides* are very similar to those of *B. allii* in shape, color, and mode of germination. (Fig. 6, O.) They are occasionally a little longer in relation to their width and thus have a more oblong appearance. They vary considerably in size (8 to 20 by 5 to 11 μ), but, as is shown in Table 1 the majority fall within the limits of 10 to 16 by 6 to 8 μ . They therefore will constantly average somewhat larger than those of *B. allii*, both upon onion tissue and when they are occasionally produced upon potato agar. Microconidia, globose and about 3 μ in diameter, are occasionally formed.



FIG. 2.—Comparison of gray-mold and mycelial neck rots. Healthy White Portugal onion sets inoculated after harvest by the injection of spores or mycelium through neck wounds, placed in standard storage crates, and left in an onion-set warehouse at Morton Grove, Ill., for eight weeks. Those in the upper row were inoculated with *Botrytis allii*. Note the characteristic gray mold on the decayed tissue. The lower five bulbs were inoculated with *B. byssoides*. Note the absence of conidia and the development of aerial mycelium and of sclerotia on the decayed tissue

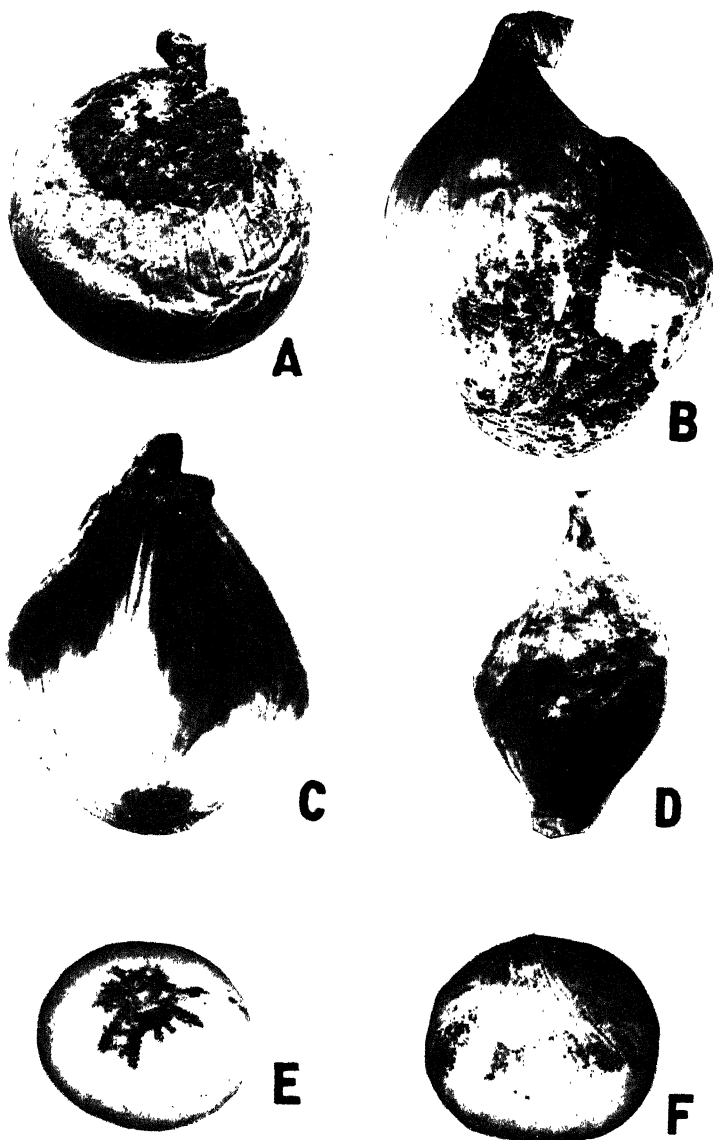


FIG. 3.—Mycelial neck rot *Botrytis byssoides* on White Globe (A to D) and White Portugal (E, F) onions. Note the formation of sclerotia on the older decayed tissue (A, B). Basal infection commonly occurs (B). The first evidence of infection of the tissue is a water-soaked appearance; the decayed tissue becomes permeated with mycelium and gradually shrivels (C). An abundance of superficial mycelium is common (D). An early stage of infection at the neck is shown in F. Occasionally rapid desiccation of newly infected scales checks the further progress of the fungus (F)

PATHOGENICITY

The mycelial neck-rot disease has been produced repeatedly by inoculating onion bulbs through needle wounds with mycelium from pure culture and with conidia and conidiophores which developed upon naturally infected or upon artificially inoculated bulbs. As in the case of *Botrytis allii*, the most uniform results were obtained when too-rapid desiccation of the wound tissue was prevented. The most satisfactory results were obtained by placing the inoculated bulbs in oiled paper bags and incubating them at 15° to 20° C. Under these conditions the characteristic bulb rot occurred, and the progress of the decay was approximately equal to that of gray-mold neck rot. Under humid conditions and favorable temperature, invasion of the unwounded outer succulent scales has been noted, and the spread from scale to scale through the unbroken cuticle has been demonstrated. Initial infection through the dry outer scales or through the unbroken outer succulent scale does not commonly occur in nature, however, and there is little evidence of natural invasion except by way of wounds or the dying tissues at the neck of the bulbs.

With respect to leaf infection, what has already been said of a gray-mold neck rot may well apply to mycelial neck rot. No evidence has been found in nature that this organism causes a serious blight of onion tops. It is commonly found fruiting upon dead or senescent leaf tissue during humid weather, but is to be regarded as little more than a saprophyte. Vigorously growing onion plants inoculated with a spore suspension of the organism and kept in a moist chamber at about 18° C. for 48 hours and removed to a greenhouse at 15° to 20° did not yield evidence of infection. As in the case of *Botrytis allii*, plants inoculated in such a manner and kept under very humid conditions at 15° to 20° for one to two weeks eventually showed evidence of leaf infection. It is questionable, however, if this situation commonly exists naturally in onion-growing regions in the United States. *B. byssoides* was found in one instance causing secondary infection of seed stems of Egyptian onion that has been infected with rust (*Puccinia asparagi*) (30). In the original report this was not considered to be a neck-rot form, since first inoculations of the bulbs gave negative results. When the proper conditions for infection were later determined, positive infection of bulbs with this strain was obtained.

HOST RANGE

Bulbs of white multiplier onion and of white shallot when inoculated with *Botrytis byssoides* decayed quite as rapidly as did those of common onion. Bulbs of garlic were also successfully inoculated, but, as in the case of *B. allii*, the progress of the disease was distinctly slower than upon the other hosts.

SMALL SCLEROTIAL NECK ROT

SYMPTOMS

The small sclerotial neck rot has so far, with one exception, been found only upon white varieties of onion. In one instance it was found in a few very slightly pigmented bulbs which appeared in a lot of Yellow Globe variety and were probably the result of previous crossing with a white variety. As will be pointed out later, colored bulbs are

readily infected when inoculated through wounds in the succulent scales. The fungus most commonly appears first near the neck of the bulb and, as a rule, not until some weeks after the crop has been harvested and stored. It is often confined to the two or three dry outer scales. At times it is found upon the succulent scales, commonly in association with the mycelial neck rot.

Usually the first evidence of the disease is the appearance of very thin, scalelike sclerotia which adhere very closely to the dry scales. The sclerotia are at first light colored, but most of them turn completely black with age. In many instances their development is arrested prematurely, and bodies which are light gray in the center and black only at the margin result. The sclerotia are usually roughly circular and measure from $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in diameter. They vary from this form, however, being sometimes larger and more irregular in shape. The mycelium of the fungus is scanty and not often distinguishable microscopically upon the affected dry outer scales. When it does appear it is scattered and has a dark-green cast. Sporulation occurs on conidiophores, arising most commonly from the sclerotia and more rarely from the scattered mycelium. At room temperature very little sporulation can be induced even when affected scales are placed in a moist chamber, but under moist conditions in the ice box or at temperatures between 5° and 18° C. sporulation upon the sclerotia is common.

The decay of the succulent scales is distinctly slower than that caused by either *Botrytis allii* or *B. byssoidea*. The cytolytic action in advance of the hyphae, characteristic of the other two forms, prevails in this case as well. The newly rotted area is water-soaked in appearance, and there is a quite definite margin between diseased and healthy tissue. In the older decayed portions grayish superficial mycelium is formed, though usually rather scantily, while sclerotia are also commonly embedded in the rotted tissue. The decayed portions of the bulb often become distinctly brownish in color, and in this respect the disease is distinct from the gray-mold and the mycelial neck rots. Certain aspects of the disease are illustrated in Figure 4.

THE CAUSAL ORGANISM (BOTRYTIS SQUAMOSA)

The mycelium of the small sclerotial form is not sufficiently different from that of the other two onion *Botrytis* forms to be of diagnostic value. Appressoria are commonly produced. The sclerotia are quite characteristic. They vary in size from $\frac{1}{2}$ to $1\frac{1}{2}$ mm. or more, and often converge into large, irregular scalelike crusts. On the host they are almost always very thin and flat and seldom acquire the thickness of $\frac{1}{2}$ mm. They are usually roughly circular, but occasionally they are quite irregular in form. They appear first as dense, thin mycelial mats of a dirty-white color. They become darker with age and gradually become black, the color appearing first at the outer margins and progressing inwardly until the whole sclerotium is deep black. Occasionally the development of color is arrested prematurely, with the result that the sclerotia have light grayish centers and black margins.

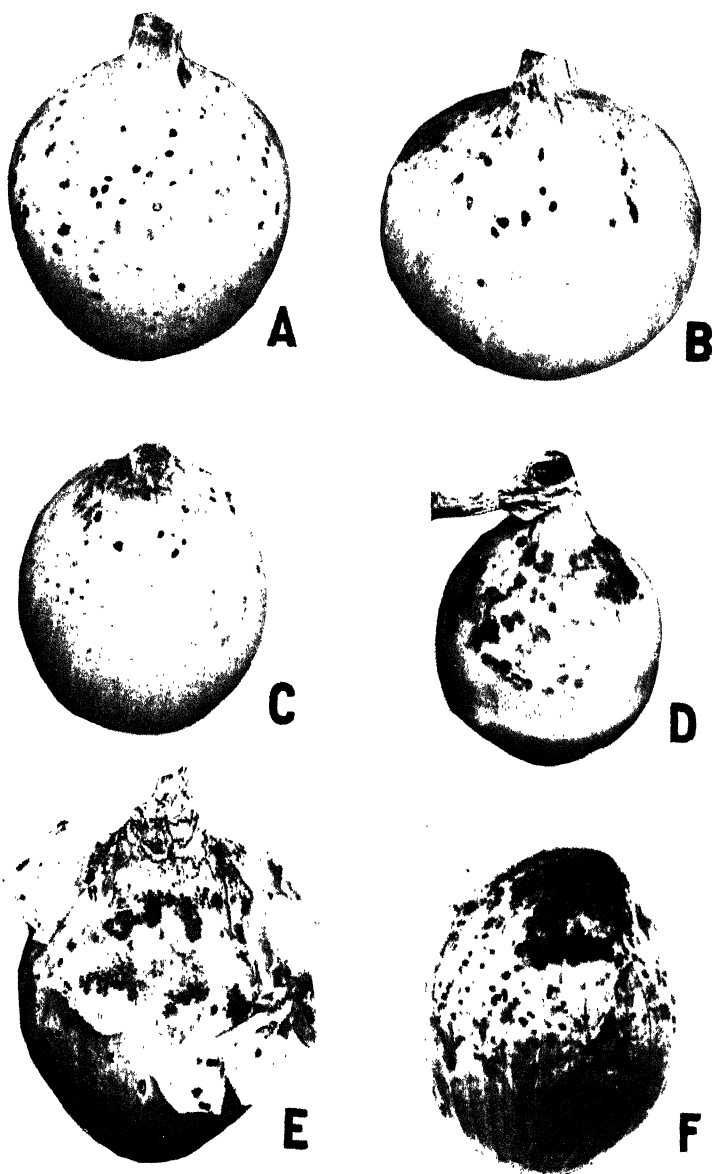


FIG. 4.—Small sclerotial neck rot *Botrytis squamosa* on White Globe onions. Thin, flat, black sclerotia develop commonly on the dry outer scales. Invasion of the succulent scales sometimes occurs (D, E). In F, *Botrytis byssoides* is also present in the succulent scales. The larger, more rounded sclerotia near the neck of the bulb are those of the latter fungus.

The conidiophores arise occasionally directly from free mycelium, but in the main are produced upon the sclerotia. They are hyaline at the growing tip, but the walls turn dark promptly as they become a little older. They are septate, and occasionally branched, the branches being constricted at the point of juncture with the main stalk. Previous to sporulation the growing tip gives rise to a number of branches which rebranch and at their rounded tips send out sterigmata upon which the conidia are borne in the same manner as described for *B. allii* and *B. byssoidea*. (Fig. 6, J.) The sporulating branches are, as a rule, shorter and plumper than those described for the other two forms. As the spores mature, the branches, having been cut off close to the main stalk by septa, rapidly degenerate. As they do so the walls of these branches contract in a unique manner and give the appearance of closing together in folds suggestive of the contracting bellows of a camera. (Fig. 6, K.) The spores, if not removed by air current or by water, adhere in a group to the sides of the main stem of the conidiophore which now proliferates by growing upward, again branching and sporulating. Scars or knobs not unlike those previously described for the other two species mark the points at which degenerating sporiferous branches are attached. Occasionally one of the branches becomes permanent.

The conidia are hyaline, ovoid, and continuous, and germinate within 24 hours under favorable conditions by each sending out usually one hyaline germ tube. (Fig. 6, L.) Septation of the spores upon germination is not common. They vary in size from 13 to 24 μ by 9 to 18 μ , but the majority of them fall within the limits of 15 to 22 by 11 to 15 μ . (See Table 1.) Microconidia, globose and about 3 μ in diameter, are occasionally formed.

PATHOGENICITY

Inoculation of healthy onion bulbs was brought about by injecting mycelium or sclerotia from pure cultures of *Botrytis squamosa* into needle wounds made near the necks. The bulbs were placed in oiled paper bags and incubated at various temperatures. The most rapid decay occurred at about 16° C., although infection did occur over a range of 4° to 22°. Even at the optimum the decay progressed much more slowly than in the case of *B. allii* or *B. byssoidea*. Results were obtained, however, which left no doubt that the organism is pathogenic upon the succulent scales and that under these conditions colored bulbs decayed quite as readily as white bulbs. Direct infection through the unwounded surface of the succulent scale occurs under proper conditions of humidity and temperature, although this does not appear to be the usual method of initial infection.

Vigorously growing onion plants were inoculated with a spore suspension of the organism and transferred at once to a saturated atmosphere held at about 18° C. After 48 hours the plants were transferred to a greenhouse kept at 15° to 20°. No evidence of infection developed. When plants were left in the moist chamber for a week or more, leaves were attacked in the manner already described in connection with *Botrytis allii* and *B. byssoidea*. *B. squamosa* was somewhat more aggressive under these conditions than were the other two species. Severely infected plants, when removed to ordinary greenhouse conditions, rapidly outgrew the disease. It appears, therefore, that in an extremely humid environment having

a favorable temperature, infection of the aerial parts by *B. squamosa* may occur. But, as in the case of the other two neck-rot organisms, there is little evidence that this form causes a destructive leaf blight, and its relation to the host during the growing season is primarily one of subsistence on dead or senescent tissue.

Infection of bulbs through the unwounded neck has not been produced by artificial inoculation, but the common observation of the disease upon bulbs regardless of whether the tops were clipped at harvest shows that the organism penetrates the scales by way of the senescent leaf tissue without the aid of artificial wounds.

COMPARATIVE STUDIES OF THE THREE NECK-ROT ORGANISMS

CULTURAL CHARACTERS

All three species of *Botrytis* associated with onion neck rot grow readily upon onion and potato-dextrose agar. On either of these media the growth characters of each species are quite distinctive, and they afford a convenient means of differentiation. It has not been found necessary to study the development of these fungi on a wide range of artificial media. Potato agar containing 2 per cent dextrose has been used as stock medium, and the salient characters of growth on this agar will be given.

BOTRYTIS ALLII (fig. 5, A)

On potato-agar plates a rapidly growing colony was produced, which attained a diameter of about 50 cm. in 4 days at 20° to 22° C. In the beginning the center of the colony consisted of dense whitish aerial mycelium, while the outer zone, about 5 mm. in width, consisted of scanty, creeping, extending mycelial threads. Conidiophores and conidia appeared on about the third day, nearly white at first, turning smoky gray with age. Eventually the entire plate became covered with a dense uniform layer of conidiophores. Sclerotia were not usually produced but occasionally developed in test-tube cultures with this medium. Dark-colored appressoria were sometimes formed when the mycelium penetrated the agar layer and came in contact with the glass plate, but here again the development was more pronounced in test-tube cultures at the edge of the slant. There was little difference in growth to be noted when maltose, lactose, or galactose was substituted for dextrose in the medium. Onion-scale agar and potato agar made without the addition of sugar produced growth quite similar to that on potato-dextrose agar, the chief point of difference being the smaller amount of appressorial development. The organism was carried for seven years on potato-dextrose agar without any noticeable change in growth characters, and this period of artificial culturing did not change the pathogenicity of the fungus.

BOTRYTIS BYSSOIDEA (fig. 5, B)

On potato-dextrose agar plates the *Botrytis byssoides* colony grew somewhat faster at 20° to 22° C. than did *B. allii*. Abundant white, fluffy, aerial mycelium was formed, which was more raised and cottony than that of either *B. allii* or *B. squamosa*. It was fairly uniform in density except at the narrow outer zone of extending

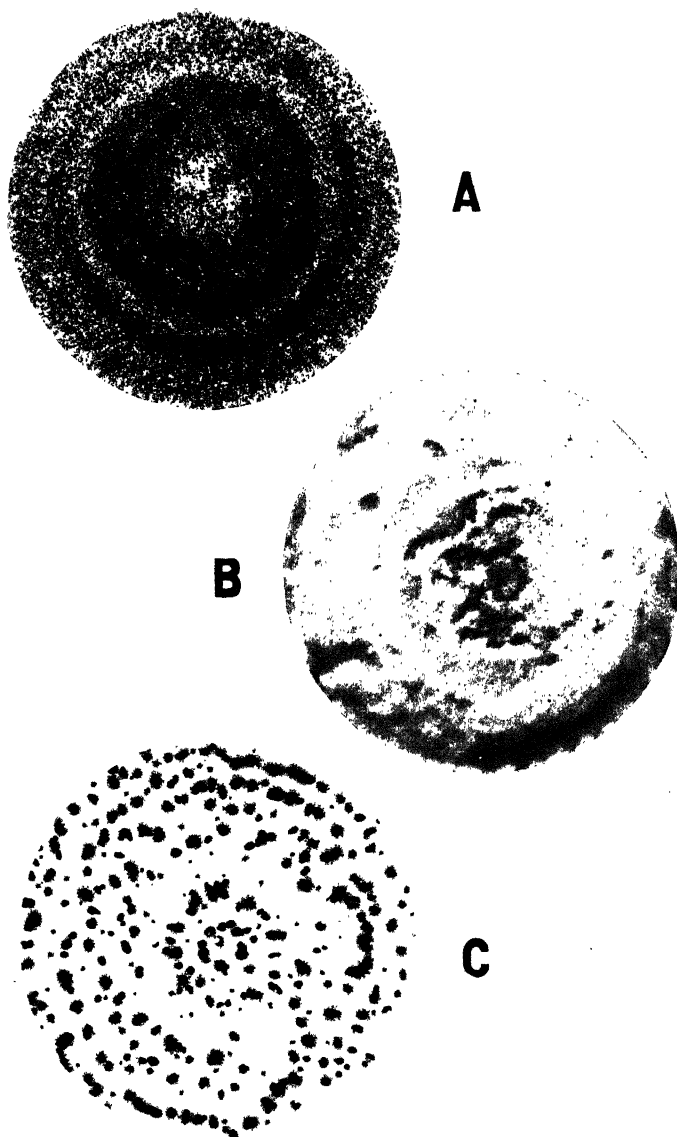


FIG. 5.—The three onion neck-rot organisms on potato-dextrose agar. A, *Botrytis allii*. Profuse production of conidia. B, *Botrytis byssoides*. Profuse mycelial development but no conidia. C, *Botrytis squamosa*. Mycelial development followed by production of sclerotia. No sporulation occurred at room temperature, 20° to 22° C. After two months the culture was removed to the ice box, where profuse sporulation took place. Note the conidiophores arising from the sclerotia

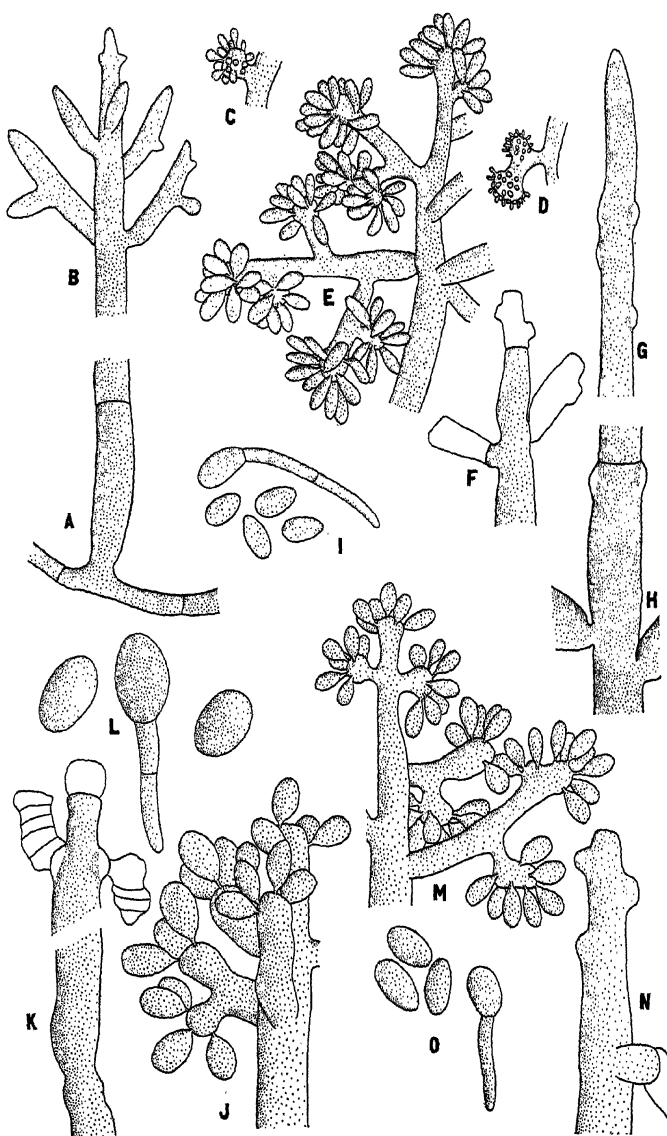


FIG. 6.—Conidial production. A to I, *Botrytis allii*; J to L, *B. squamosa*; M to O, *B. byssoides*. Conidiophores arise from mycelium (A) and branch previous to sporulation (B). The sporiferous tips become rounded and send out numerous sterigmata (D) from which growth continues (C) to produce conidia (E). When the spores are mature the branches degenerate (F) and the main stalk proliferates (G). Groups of sporiferous branches are thus formed several times leaving scars on the main branch (G) in each case. Sometimes the branches become permanent (H). The process is similar in each species (J, K, and M, N). Note characteristic accordionlike folds in degenerating sporiferous branches of *B. squamosa* (K). See further explanation in text.

hyphae. Conidia were seldom produced. Appressorial development was pronounced at the edge of test-tube slant cultures. Sclerotia were usually absent in both plate and tube cultures grown at room temperatures. In young tube cultures placed out of doors on January 25, 1926, at Madison, Wis., numerous characteristic sclerotia had formed when they were brought in on March 19. In another culture, about a month old when placed out of doors on December 5, 1925, and brought in on March 19, 1926, sclerotial formation did not take place but conidial production was quite pronounced. When maltose, lactose, or galactose was substituted for dextrose no conspicuous change in growth characters resulted. On onion agar and potato agar without dextrose the mycelial production was somewhat less profuse. Whereas *B. allii* was carried on potato-dextrose agar indefinitely without loss of pathogenicity, *B. byssoides* usually lost entirely its pathogenicity within a few months under such treatment, especially when kept at room temperatures (20° to 22°). This change was accompanied by a reduction in the vigor of the culture as exhibited by less profuse aerial mycelium and less appressorial development.

BOTRYTIS SQUAMOSA (fig. 5, C)

On potato-agar plates the *Botrytis squamosa* colony enlarged more slowly than either *B. allii* or *B. byssoides*. It consisted of white, fluffy, aerial mycelium, except for the outer advancing zone, but was less raised and fluffy than *B. byssoides*. Numerous sclerotia appeared over the entire plate after two to three weeks, first as dense whitish mycelial masses which became hard and black with age. They were mostly 1 to 2 mm. in width and thicker and more rounded on the upper surface than those which developed upon the host. There was very little sporulation at room temperature (20° to 22° C.), but profuse production of conidiophores arising directly from the sclerotia occurred at 12° to 16°. Substituting maltose and lactose for dextrose made little difference in the growth produced. When galactose was used, however, the sclerotia assumed a distinctly different form. They were a little larger, more irregular in shape, very thin and flat, and embedded just at or slightly below the surface of the substrate. On onion agar and potato agar made up without the addition of sugar fewer sclerotia were produced.

OVERWINTERING AND SEASONAL CYCLES

Experiments were undertaken to determine by what means the three neck-rot organisms might live through the winter at Madison, Wis. Sclerotia and spores on bulbs affected by mycelial and gray-mold neck rot were exposed to prevailing weather from December to March in the winter of 1925-26 by placing them in wire baskets on an outer window sill. Both sclerotia and spores of each organism were viable at the end of this period. Pure cultures were exposed over the same period, and they also remained viable. A culture of the small sclerotial neck-rot organism containing sclerotia and conidia was also exposed. Transfers from sclerotia as well as from the conidia and conidiophores were made at the end of the period with positive results in each case. It is thus apparent that the sclerotia will withstand winter weather, while it is not improbable that the mycelium and spores under certain conditions may also live through the extremely cold portion of the year.

From general field observations it is quite evident that each of the organisms persists from year to year in the soil or on refuse. The organisms from such materials are undoubtedly the beginning of saprogenic development the following season, when they live as saprophytes upon dying onion leaves and other organic matter during the growing season of the host. Spores from this saprogenic phase of the organisms are the source of inocula for bulb infection at and after the end of the growing season.

TEMPERATURE RELATIONS

GROWTH ON MEDIA

The relation of temperature to the range and rate of growth of the three organisms was studied upon potato-dextrose agar. Separate slants were inoculated each with one of the three organisms and placed in constant-temperature incubators the temperature of which ranged from 3° to 33° C. The three organisms responded quite similarly to the various temperatures. Growth ensued very slowly at 3° to 4°. There was a gradual increase in the rate of growth with increase in temperature up to about 20° while the rate was about the same between 20° and 25°. At 28° there was decidedly less growth than at the optimum, and at 30° there was still greater retardation. At 33° there was some growth of each organism, but it was very meager in all cases. Thus all three forms grew upon potato-dextrose agar within the range of 3° to 33°, the growth being very slow at either end of the range and most rapid at about 20° to 25°.

SPORE GERMINATION

Germination tests were conducted at various temperatures with the conidia of each of the three species. Spore suspensions were made in tap water and drops transferred to clean glass slides, which were placed in moist chambers in the incubators. In Table 2 the rate of germination in each incubator is given. Spores of *Botrytis squamosa* germinated more promptly than those of the other two species. It is to be seen that germination occurred more promptly at temperatures between 19° and 27° C. than at temperatures below 19°, but, on the other hand, it took place over the entire range of temperature with each species after a period of 24 hours.

TABLE 2.—Relation of temperature to spore germination^a of *Botrytis allii*, *B. byssoidea*, and *B. squamosa*

Temperature °C.	Species and interval											
	<i>Botrytis allii</i>				<i>Botrytis byssoidea</i>				<i>Botrytis squamosa</i>			
	4 hours	8 hours	12 hours	24 hours	4 hours	8 hours	12 hours	24 hours	4 hours	8 hours	12 hours	24 hours
3.5-4.....	0	0	0	+	0	0	0	+	0	0	0	+
6.5-7.....	0	0	0	+	0	0	0	+	0	0	0	+
8.5-10.....	0	0	0	+	0	0	0	+	0	0	0	+
13.5-15.....	0	0	+	+	0	0	+	+	+	+	+	+
16.5-18.....	0	0	+	+	0	0	+	+	+	+	+	+
19.5-20.....	0	+	+	+	0	+	+	+	+	+	+	+
22.5-23.....	0	+	+	+	0	+	+	+	+	+	+	+
24.5-25.5.....	0	+	+	+	0	+	+	+	+	+	+	+
26.5-27.....	0	+	+	+	0	+	+	+	0	+	+	+

^a + indicates germination; 0 indicates no germination.

SPORULATION

It is of course important to know the bearing of temperature upon the production of spores of the fungi when exposed to properly humid conditions. *Botrytis allii* appears to sporulate readily at comparatively low relative humidity, but that the other two forms do is not so evident. *B. allii* sporulates readily in culture or upon rotted onion tissue over a range of 4° to 25° C. *B. byssoidea*, although it does not sporulate readily in culture, will do so when old decayed onion tissue is placed in a moist chamber. Under these conditions *B. byssoidea* produces conidia most quickly at about 23° but quite promptly within a range of 13° to 27°. At temperatures between 3° and 13° it sporulates more slowly. *B. squamosa* has a more limited range of sporulation. No conidia have been found at 22° either in pure cultures or on decayed tissue kept in a moist chamber. The range at which sporulation ordinarily occurs under these conditions is about 18° to 3°. The optimum is about 16°.

INFECTION AND PROGRESS OF DECAY

A number of experiments were made to determine the relation of temperature to infection and the rate of progress of decay. The bulbs were all inoculated by injecting mycelium or spores into needle wounds near the neck. They were then placed either in oiled paper bags or in uncovered paper boxes and incubated at different temperatures. The first two experiments are concerned with *Botrytis allii* and *B. byssoidea*. In the first experiment, the results of which are given in Table 3, the bulbs were all placed in oiled paper bags.

TABLE 3.—The relation of temperature to infection and progress of decay of onion bulbs inoculated with *Botrytis allii* and *B. byssoidea*; five bulbs in each lot; Red Wethersfield variety

Temperature °C.	<i>Botrytis allii</i>		<i>Botrytis byssoidea</i>		Control
	Per cent infected	Rate of progress ^a	Per cent infected	Rate of progress ^a	Per cent infected
2-4	90	1	100	1	0
5-8	100	2	100	2	0
10-11	100	3	100	3	0
15.5-16.5	100	4	100	4	0
18.5-19.5	100	4	100	4	0
23-23.5	100	3	100	3	0
26.5-27.5	0	-----	20	1	0
28.5-29.5	20	1	20	1	0
32-34	0	-----	0	-----	0

^a 1 indicates the least rapid and 4 the most rapid decay.

The temperatures below 20° C. were maintained in an incubator where the humidity was quite high; but in the incubators at higher temperatures the humidity was correspondingly lower, and the oiled paper bags did not entirely prevent the desiccation of the decaying bulbs. Thus the possible influence of humidity as a limiting factor must be considered. It is to be seen that in this experiment the most rapid decay occurred at 15° to 20°, while above 26° the amount of infection and the rate of decay were decidedly limited.

Another series of experiments was run to determine whether infection would be accelerated at the higher temperatures if a high relative humidity prevailed. Two chambers in which the temperature was 25° to 27° and 28° to 30°, respectively, were kept at saturation. Inoculated bulbs were placed in open boxes in each. At the same time boxes of inoculated bulbs were placed in other chambers having different temperatures and different relative humidities. In the latter series the environment was not controlled as accurately as was desired, but the average temperatures and relative humidities as recorded on thermographs and hygrographs are given, and the results are included for their comparative value. (Table 4).

TABLE 4.—The relation of temperature and humidity to decay of onion bulbs by *Botrytis allii* and *B. byssoidea*; 10 bulbs in each lot; Red Wethersfield variety

Temperature °C.	Relative humidity per cent	<i>Botrytis allii</i> , per cent infected	<i>Botrytis byssoidea</i> , per cent infected	Control, per cent infected
10° ±	60 ±	100	100	a 10
15° ±	50-75	100	100	0
15°-20°	75-85	100	100	0
22°-25°	60-80	100	100	0
22°-27°	60-75	70	80	0
25°-30°	75-85	70	20	0
25°-27°	100	100	100	0
28°-30°	100	0	0	0

a One of 10 bulbs became infected by *Botrytis allii* during the experiment.

At temperatures below 20° C. the results were similar to those in the previous experiment. At temperatures above 20° the relative humidity was higher than in the previous experiment. Here it is evident that the higher humidity accelerated infection, although as was noted before, the progress of decay was distinctly retarded as the temperature rose above 22°. A comparison of the lots held at 100 per cent relative humidity at 25° to 27° and 28° to 30° shows that the maximum temperature for infection is somewhere between 27° and 30°. It is also significant that throughout these experiments *Botrytis allii* and *B. byssoidea* reacted very similarly to temperature. In Figure 1 are shown the relative amounts of decay in bulbs inoculated with *B. allii* and *B. byssoidea*, respectively, and held in moist chambers at 18°, 23°, and 26°.

Two experiments of a similar nature were run with *Botrytis squamosa*. The bulbs were placed in oiled paper bags in an incubator at temperatures ranging from 23° to 4° C. Infection occurred over the entire range. The most rapid decay took place at about 15°. At 18° it was nearly as rapid as at 15°. At 23° it was distinctly retarded, but desiccation was somewhat greater than at the lower temperatures. Below 15° there was a gradual slowing up of activity roughly proportionate to the reduction in temperature.

RECAPITULATION

The data which have just been noted point to the conclusion that all three fungi are favored by temperatures between 15° and 20° C. It is true that mycelial development is somewhat more rapid and

conidial germination a little prompter between 20° and 25°, but on the other hand the infection studies show quite clearly that the diseases progress less rapidly above 20° than between 15° and 20°. Sporulation in *Botrytis allii* and *B. byssoidea* occurs over a wide range if the atmosphere is humid, and this holds true even in the saprophytic stage of the fungi during the growing season of the host. *B. squamosa* is more limited in this respect, inasmuch as there appears to be little or no sporulation above 20°.

It may thus be assumed that humid cool weather during the growing season of the onion plant is most favorable to the development and spread of the neck-rot fungi, while hot dry weather is correspondingly less favorable. The small sclerotial neck-rot organism is probably the one most retarded by higher temperatures, and this belief is supported by the fact that it is usually not found in midwestern districts until some weeks after the appearance of mycelial neck rot. At harvest time, when infection of the bulbs commonly occurs, warm dry weather is least favorable to infection, and in fact incipient infection of mycelial neck rot has often been found to be completely checked by such an environment.

As substantiating these views some evidence from field observations on the occurrence of mycelial neck rot over a period of years in southeastern Wisconsin may be cited. In this district two severe epiphytotics have occurred in the decade from 1915 to 1924. In 1915 the disease was very severe even in the resistant Red Globe variety, while in 1924 the damage to this variety was nearly as great, and with the white varieties it approached a complete loss. In contrast with these two years, the season of 1919 was one of great scarcity of neck rot. Climatically, 1915 and 1924 represent one extreme and 1919 the other. An examination of the weather records taken by the United States Weather Bureau at Racine, Wis., give some tangible details. The season of 1915 was one of abundant rainfall, the total precipitation from June 1 to September 30, inclusive, being 15.76 inches, which was 2.92 inches above normal. The mean temperatures for June, July, and August were, respectively, 3.8°, 4.3°, and 4.4° F. below normal, while that for September was 1.4° above normal. In the season of 1924 the precipitation was 6.76 inches above normal, while the mean temperatures of the four months were, respectively, 2.2°, 0.7°, 1.3°, and 4.3° below normal. In 1919 the precipitation for the four-month period was only 0.17 inch above normal, while the mean temperatures were 5.9°, 4.9°, 1.4°, and 3.2° above normal. During the 1915 and 1924 periods there were, respectively, 77 and 49 days of cloudy or partly cloudy weather, while in 1919 there were only 37 such days. Thus the two epiphytotic years were unusually moist, cool, and cloudy during the major portion of the onion-growing season; while the year of least disease of the decade was unusually dry, warm, and clear during the corresponding period.

It is to be expected, from what we know of the life history of the *Botrytis* forms, that moist, cool, cloudy weather is most favorable to the saprophytic development of the organism during the growing season of the onion. The same conditions are noticeably effective in delaying the maturity of the crop and tend to develop plants

which are more succulent at the neck and thus slower to desiccate at the point where *Botrytis* infection occurs. Furthermore, such weather is most conducive to infection at the critical time. This fact was most noticeable in 1915 when maturity was delayed some three weeks, and the frequent rains during the harvest period made proper curing almost impossible. In 1924 the delay of harvest was not so great, but careful examination showed the organism to be widespread and fruiting abundantly on old leaves and the dying tips of the leaves just previous to pulling of the crop. The clear, hot, drying weather of 1919, on the other hand, was unfavorable to the multiplication and spread of *Botrytis* during the season, a fact which tended to reduce the amount of natural inoculum at harvest time. Moreover, the maturity of the host plant was hastened under such conditions, and the top growth was so reduced as to facilitate rapid and thorough desiccation of the neck. It seems quite likely that the influence of climatic conditions is exerted in three important directions: First, they create conditions favorable or unfavorable to the multiplication of the parasite; second, they prolong or shorten the period of growth of the host and influence the degree of succulency of the neck at harvest time; third, they influence infection at the critical time by either favoring or retarding the germination and growth of the causal organism.

RELATION OF STAGE OF MATURITY OF HOST TO INFECTION OF BULBS

It has already been intimated that the condition of the host tissues at the time of exposure to the parasites is important in determining infection. Some laboratory experiments will be reported which were designed to study this point.

LABORATORY EXPERIMENTS WITH *BOTRYTIS ALLII*

EXPERIMENT 1

Three varieties of onion, White Portugal, Yellow Globe, and Red Globe, were grown on soil which had not produced this crop for at least five years. When the tops of the plants had fully matured the bulbs were pulled. Two groups from this lot of bulbs were prepared. In one group tops were left intact; in the other they were clipped sufficiently near the bulb to expose the succulent tissues of the scales. The two groups were again divided into equal parts, one part in each case being used as a control, the other for inoculation. The controls were sprayed with sterile water, while the remaining bulbs were sprayed with a conidial suspension of *Botrytis allii*. The controls and the inoculated bulbs were then placed in separate wire baskets and kept in a room having a fairly constant temperature of about 18° C., where they were suspended in a partially closed chamber over moistened sphagnum moss. The inoculations were made on September 3, and the bulbs were examined on September 22. The final data are noted in Table 5.

TABLE 5.—*Development of Botrytis allii in topped and untopped bulbs of three varieties of onion; experiment 1*

Variety	Method of handling tops	Inoculated		Control	
		Total number of bulbs	Number with neck rot	Total number of bulbs	Number with neck rot
White Portugal.....	Topped.....	20	17	20	0
Do.....	Untopped.....	20	0	20	0
Yellow Globe.....	Topped.....	10	8	10	0
Do.....	Untopped.....	10	1	10	0
Red Globe.....	Topped.....	10	5	10	0
Do.....	Untopped.....	10	0	10	0

The outstanding result is that whereas infection took place quite readily when the succulent tissue was exposed, only 1 bulb out of the 40 which were exposed to the inoculum with the tops intact became diseased.

EXPERIMENT 2

The experiment reported above was repeated with some modifications. Red, Yellow, and White Globe plants were pulled, and the tops were allowed to cure out thoroughly. They were then divided into four groups and prepared for inoculation in the following ways: (1) The tops were cured down but not removed; (2) 1 to 2 inches of thoroughly cured neck were left intact; (3) no neck tissue was left, but since the tops were thoroughly cured down to the bulb proper no succulent tissue was exposed; (4) the necks were so removed as to expose wounded, succulent tissue. A portion of each lot was then sprayed with a conidial suspension of *Botrytis allii*; a second portion was sprayed with conidial suspension and covered with moistened filter paper; a third portion was sprayed with sterile water and covered with moistened filter paper to serve as a control. All three lots were stored in shallow trays at about 18° C. The inoculations were made on September 23. The final observations, made on November 27, are recorded in Table 6.

TABLE 6.—*Relation of neck tissue to infection of Botrytis allii in bulbs of White, Yellow, and Red Globe varieties; experiment 2*

Condition of neck tissue	Variety	Inoculated and not covered		Inoculated and covered with moistened filter paper		Sprayed with sterile water and covered with moistened filter paper	
		Number of bulbs	Number with neck rot	Number of bulbs	Number with neck rot	Number of bulbs	Number with neck rot
Untopped.....	White.....	25	0	27	1	26	0
	Yellow.....	25	0	27	0	25	0
	Red.....	25	0	26	0	25	0
1 to 2 inches of the neck left intact.....	White.....	25	0	25	0	25	0
	Yellow.....	49	0	27	0	3	0
	Red.....	29	0	26	0	28	0
No neck tissue left; succulent tissue not exposed.....	White.....	25	0	25	0	17	0
	Yellow.....	25	0	25	0	27	0
	Red.....	25	0	27	0	24	0
Tops clipped so as to expose wounded tissue.....	White.....	25	9	23	11	25	0
	Yellow.....	25	6	29	15	25	0
	Red.....	25	1	30	13	25	0

It is again evident that the desiccated neck tissue is, under the conditions of the experiment, an effective barrier against the invasion of *Botrytis allii*.

LABORATORY EXPERIMENT WITH BOTRYTIS BYSSOIDEA

EXPERIMENT 3

With bulbs from the same lot used in experiment 1 a comparable experiment was conducted at the same time, wherein inoculations were made by spraying with a conidial suspension of *Botrytis byssoides*. Inoculation was made on September 3 and the final data were recorded on September 22. (Table 7.)

TABLE 7.—Development of *Botrytis byssoides* in topped and untopped bulbs of three varieties of onion; experiment 3

Variety	Condition of tops	Inoculated bulbs		Control bulbs	
		Total number	Number with neck rot	Total number	Number with neck rot
White Portugal.....	Topped.....	21	19	20	0
Do.....	Untopped.....	19	3	20	0
Yellow Globe.....	Topped.....	10	8	10	0
Do.....	Untopped.....	10	1	10	0
Red Globe.....	Topped.....	10	8	10	0
Do.....	Untopped.....	10	0	10	0

It is evident here, as with *Botrytis allii*, that the removal of the top and exposure of the wounded succulent tissue favors infection. In this instance a few more bulbs with tops intact were infected than was the case with *B. allii*. The difference is not great enough, however, to warrant the conclusion that *B. byssoides* is more aggressive as an invader through the uninjured neck tissue, although this question deserves further study.

It is to be expected that inception of the neck-rot diseases in the field may be influenced by the condition of the neck tissues of the bulb when it is exposed to the parasites under favorable environment. In the experiments just described the bulbs were not exposed to the organisms until the tops were entirely mature. In the light of this evidence it might be expected that bulbs which were thoroughly mature before being subjected to the organisms would remain reasonably free from infection. The opportunity for prompt and thorough desiccation of the neck tissues does not always prevail in the field. In general those conditions which favor sporulation of *Botrytis allii* and *B. byssoides* and subsequent germination of conidia are the ones which inhibit rapid curing of the onion tops. It remained for actual field experimentation to determine whether or not the best natural curing of bulbs with the tops left intact was sufficient to ward off infection by the neck-rot fungi.

FIELD DATA

The field studies were carried on in the onion-growing section of eastern Racine County, Wis. An area was selected where the crop had been grown repeatedly for many years and where mycelial neck rot commonly occurs on the stored crop. Unfortunately for this

study, the gray-mold neck rot occurs only rarely in this section. The small sclerotial neck rot sometimes occurs, but it is confined to the white varieties, and they are grown to a very limited extent in this section. The field observations are therefore confined almost entirely to *Botrytis byssoides*.

The first series of experiments was planned to compare the amounts of neck rot which developed in bulbs with mature and with immature necks at harvest. It was necessary to harvest such bulbs from practically the same spot and to handle them in the same manner in order to give equal exposure to natural inoculum and environing influences. Consequently a portion of a field was selected in which the crop was approaching maturity and in which the plants were ripening somewhat unevenly. The bulbs were harvested and divided into two groups designated as "mature" and "immature" according to the succulence of the neck tissues. They were then topped and placed in slatted crates, where they were allowed to cure in the open for some weeks, after which they were stored in an onion warehouse. The data obtained at the end of the storage periods are given in Table 8.

TABLE 8.—*Relation of maturity of neck tissue to natural infection of Red Globe onions by Botrytis byssoides*

Year	Immature		Mature	
	Total number of bulbs	Percentage with neck rot	Total number of bulbs	Percentage with neck rot
1917.....	600	6.5	600	2.8
1918.....	363	21.2	661	8.6

Although the percentage of infected bulbs in the mature lots was small in both years, the amount was at least doubled in each case in the immature lots. These results are in line with what might be expected from the foregoing laboratory data.

The next matter of importance was to determine whether leaving the tops intact after harvest is a reliable barrier against infection. The possibility that it is, is suggested by the laboratory experiments, inasmuch as clipping the tops while only partially mature exposes the succulent wounds to natural inoculum. It is quite possible, however, that the fungus may become established in the dying tops before they are completely desiccated and thus gain ready access to the scales. Field experiments over a period of four years give a fair index as to the bearing of this point of natural infection. In each season from 1917 to 1920, inclusive, several bushels from the same area were harvested with and without tops. They were handled otherwise like the commercial crop. In 1917 and 1918 Red Globe variety was used. In 1919 White Globe and Red Globe varieties were used, but no neck rot developed in either lot; hence no report is included in the table. In 1920 Red, White, and Yellow Globe were used. In the last season two harvests were made. The first was on September 6, when the tops were not quite mature; the second was on October 9, some three weeks after harvest would normally occur, during which time they were exposed to some rainy weather. The percentage of neck-rotted bulbs was determined at the end of the storage period, and the data are recorded in Table 9.

It is again evident that the desiccated neck tissue is, under the conditions of the experiment, an effective barrier against the invasion of *Botrytis allii*.

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TABLE 9.—*The effect of the removal of onion tops upon infection by Botrytis byssoides; experiments at Racine, Wis., 1917-1920; data at end of the storage period*

Year and variety	Topped bulbs		Untopped bulbs	
	Total number	Percentage with neck rot	Total number	Percentage with neck rot
1917:				
Red Globe.....	1, 228	5. 1	1, 164	0. 6
1918:				
Red Globe.....	292	8. 2	292	2. 7
1920 (harvest on Sept. 6):				
Red Globe.....	243	0. 0	194	0. 0
Yellow Globe.....	224	0. 0	214	0. 0
White Globe.....	296	40. 2	340	37. 3
1920 (harvest of Oct. 9):				
Red Globe.....	340	0. 2	365	0. 6
Yellow Globe.....	323	5. 5	297	0. 0
White Globe.....	338	55. 6	304	51. 9

The data show the striking difference in susceptibility between colored and white varieties, a matter which will be discussed later. It is evident also that the bulbs in the earlier harvest, even though the tops were not quite mature at the time of harvesting, acquired less neck rot than did those which were harvested later, when the tops were mature. This is contradictory to the data previously discussed, in which mature and immature plants harvested at the same time showed the opposite results as to amount of infection. The bulbs harvested in early September had the advantage of good curing weather, which is also unfavorable for production of *Botrytis conidia* and infection. Those bulbs which remained longer in the field were exposed to environmental conditions more conducive to neck-rot infection. This is evidence therefore that the weather conditions are quite as important as the degree of maturity. Considering the effect of the removal of tops, it is evident that the bulbs of the white variety with tops left intact were affected nearly as severely as those which were topped. In the laboratory experiments (experiments 1, 2, and 3) the neck tissue of the bulbs was quite thoroughly desiccated before it was exposed to the organism. In the field this is not necessarily the case, and the difference in the field and laboratory results can only be explained by the supposition that infection in the field took place before the tops were completely cured. It is further evident that the harvesting of bulbs with the tops intact can not be relied upon as an effective means of control with the white varieties.

VARIETAL RESISTANCE

The fact has become well-established among onion growers and dealers that, of the varieties which are commonly grown for storage, the colored-bulb types have much better keeping qualities than the white-bulb type. The degree of susceptibility of the different varieties to the three neck-rot decays also tends to substantiate this fact. Munn (20) points out this difference in varietal susceptibility to *Botrytis allii*. In the Middle West it has been repeatedly observed in connection with *B. byssoides*, and the results of a critical comparison are given in Table 9. Up to the present, *B. squamosa*

has been found in nature only upon white bulbs or (in one instance) on bulbs having a very slight yellowish color. The high resistance of yellow and red bulbs is shown by the fact that in several cases where the small sclerotial neck rot occurred on white onions, colored bulbs grown next to them were completely free from the disease.

There is abundant evidence, however, that colored bulbs are not resistant once infection is established. In nature they are not always completely free from the gray-mold or mycelial neck rots. The difference between the amount of disease in colored and white varieties is usually striking, but such colored bulbs as become infected decay quite as readily as the white bulbs. Repeated comparative inoculations have been made with each of the three neck-rot organisms upon red, yellow, and white bulbs. The results have uniformly shown that infection by way of wounds in the succulent scales is attained quite as readily in one variety as in another, while the progress of decay following infection is approximately the same in colored and white bulbs inoculated with any one of the three forms of neck rot. Resistance in the colored bulbs appears to be due to the exclusion of the fungi. The outer scales and the neck tissue of pigmented bulbs contain a water-soluble substance which is decidedly toxic to the *Botrytis* forms as well as to certain other onion-bulb pathogenes. It has been suggested that this toxin may prevent the invasion of the neck tissue by the *Botrytis* organisms and thus preclude infection. Further details of this phase of the investigation have been presented earlier (32, 35).³

CONTROL

The control of the neck rots of onion has not been completely worked out as yet. The wider use of colored varieties has served to reduce the losses in a considerable measure. The greatest hazard is encountered when the white varieties are grown. There is little in the way of crop rotation and sanitation with an intensively produced crop like the onion which can be relied upon to reduce the disease in environments favorable to it. The most hopeful measure so far devised is that of artificial curing of the bulbs after harvest to check the organisms in severely infected lots. The investigations, which so far have dealt primarily with mycelial neck rot, show that rapid desiccation of the neck tissues, even though infection has already become evident, will to a great extent prevent further advance of the disease. Preliminary results have already been published (33), and further experimental work is under way.

SUMMARY

Three closely related but distinct neck-rot diseases of onion bulbs have been described. They are distinguished as follows: Gray-mold neck rot (*Botrytis allii* Munn), mycelial neck rot (*B. byssoidea* Walker), and small sclerotial neck rot (*B. squamosa* Walker).

The symptoms of each disease have been described and the morphology and pathogenicity of each organism discussed.

The three organisms are readily distinguished by the character of their growth upon potato-dextrose agar.

³ In the papers cited *Botrytis byssoidea* is referred to as *Botrytis* sp. 110 and *B. squamosa* is referred to as *Botrytis* sp. 108a.

All three organisms will produce growth on potato-dextrose agar over a range of 3° to 33° C., with most profuse development from about 20° to 25°. Spore germination occurs over a range of 3° to 27° but most promptly from about 19° to 27°. *Botrytis allii* and *B. byssoidea* sporulate at temperatures from 4° to 25° or higher, but *B. squamosa* seldom produces spores at temperatures above 20°. Infection and decay of the bulbs, however, are clearly favored most by temperatures below 20° and above 15°.

Field observations in the Middle West over a period of years show that *B. byssoidea* at least has been most prevalent during seasons in which the temperature was, on an average, below normal and the rainfall above normal.

Infection by *Botrytis allii* and *B. byssoidea* takes place more readily when the neck tissue is succulent at the time of exposure than when it has become desiccated. When harvested under comparable conditions, bulbs with immature tops showed a higher percentage of infection by *B. byssoidea* than did bulbs with mature tops. The removal of tops at harvest predisposes the bulbs somewhat more to infection by *B. byssoidea*. The difference between results with topping the bulbs and harvesting them with tops intact is not great enough, however, to warrant recommendation of the latter procedure as a means of control.

Colored varieties are in general less subject to attack by these three neck-rot organisms than are white varieties. When infection once occurs, however, decay proceeds with equal rapidity in both types. It is suggested that the water-soluble toxin present in the dry outer scales and neck tissue of colored bulbs aids materially in excluding the organisms.

The disease may be controlled in a large measure by the use of colored varieties. Artificial curing of the bulbs after harvesting so as rapidly to desiccate the neck tissues is also effective in checking the disease.

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FUSARIA CAUSING BULB ROT OF ONIONS¹

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INTRODUCTION

Immediately upon institution of cooperative work in 1917 between the fruit and vegetable inspection service of the Bureau of Agricultural Economics and the Bureau of Plant Industry, it was found that a bulb rot is an important factor in the market and transit losses of onions. *Fusarium* species were found associated with this rot. Reports of the disease in the field had been made by Selby (8)² from Ohio in 1910, by Hanzawa (5) from Japan in 1914, and by Clinton (3, 4) from Connecticut in 1915.

In the fall of 1920, the attention of the senior writer was called to serious field losses in the onion fields near Grand Junction and Delta, in the Uncompaghere Valley of Colorado. It was found also that during the previous winter exceedingly heavy losses had occurred during storage of onions in this section. The disease appeared to be like the bulb rot which had been noted repeatedly on the market in imported and domestic onions from various parts of the United States (6). Material was collected in the Colorado fields, and this became the basis for intensive study of the disease.

During 1921 the bulb rot was a serious factor in onion shipments from Texas. Some rot was also noted on the market in shipments from Colorado. During 1922 material was collected in the fields near Stockton, Calif., Walla Walla, Wash., and again in the Uncompaghere Valley of Colorado. It was found at Walla Walla that the bulb rot had been becoming an increasingly serious factor in the production and marketing of onions (2). In this section the onion crop matures in the summer. The disease not only cuts the crop short by causing premature death of the tops and small-sized bulbs but also necessitates heavy culling and immediate marketing under refrigeration to reduce losses from development and spread of the rot after harvest. Much material was collected in Walla Walla by the senior writer, and later more was sent from there by G. R. Isaman and Carl Dysart.

After the writers had begun work on the onion bulb-rot problem, it developed that J. C. Walker (12) was carrying on studies of a bulb rot of onions which occurred in the onion-set producing sections near Chicago. It was agreed that he should continue his studies of the Illinois bulb rot and that the writers would pursue their studies of the material which they had collected.

The results reported in this paper are based on studies of material collected in the markets of the United States, especially in Chicago since 1917, and in the field, especially in Colorado and Washington.

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² Reference is made by number (italic) to "Literature cited," p. 951.

Two hundred isolations have been made from affected bulbs. Single-spore cultures have been made from one-half of these. The distribution of the sources of these cultures is as follows: California (Stockton), 10; Colorado (Delta), 33; Illinois, 6; Egypt, 2; Spain, 4; Texas, 6; and Washington (Walla Walla), 131. To begin with, single-spore cultures were made of all material as it was received, and these were used for cultural and inoculation experiments. It was found impossible to continue the work on this scale, and as a result the collections made since 1923, with a few exceptions, have not been grown as single-spore cultures, and cultural and inoculation studies have been limited to about 100 single-spore cultures.

The cultures derived from single spores were grouped as much as possible on the basis of macroscopic characters. One group of 26 organisms and a few individual cultures from other groups were used for intensive work. It was found that even this one group of apparently similar organisms contained more than one species.

The situation is comparable to the potato *Fusarium* problem, and much work remains to be done. The major difficulty lies in the taxonomy of the form genus *Fusarium*, (1) which is fully appreciated only by those who have done more than desultory work with these fungi. Clearly, it is not advisable to defer publication until all the organisms collected are studied and identified.

This paper reports the results of an intensive study of the cultural characters and pathogenicity of 10 organisms whose final classification was made at the *Fusarium* conference, with the help of H. W. Wollenweber (17) and C. D. Sherbakoff.³ It is hoped that this work can be continued so as to clear up the onion *Fusarium* situation.

THE DISEASE

On the market and in storage, bulb rot of onions has been found in different degrees of severity in onions from practically all large onion-growing sections of the United States. It occurs also in onions from Egypt and Spain. It has been found that there is a definite correlation between abundance and severity of the disease in transit and storage, and in the field. This was clearly brought out by the Walla Walla situation. The fact that *Fusarium* species are soil inhabitants occurring all over the world makes it probable that *Fusarium* bulb rot will be found wherever temperature conditions are favorable for infection after onion culture has been carried on long enough to give rise to an abundant source of inoculum in the soil.

Walker and Tims (12, p. 683-684) give the following description of the disease as it occurs in the field in the Chicago region and in the greenhouse:

A progressive yellowing and dying back from the tips of the leaves is the first sign. The rapidity of this development will vary, sometimes the aerial part dying completely within one or two weeks and in other cases the decay extending over a much longer period. Appearance of newly affected plants may continue until harvest. When the early signs of the disease appear above ground, decay has already started at the stem plate. (Fig. 1, A.) The roots commonly turn pink and gradually decay, until eventually the entire root system may disappear. A semiwatery decay affecting all the tissues of the succulent scales starts from the base upward. (Fig. 1, A, B, C.) In early infections this decay may continue so as to almost completely destroy the bulb by harvest. (Fig. 1, B.) In

³ The *Fusarium* conference was held at Madison, Wis., in 1924. The writers are indebted to the following who took part in it: H. W. Wollenweber, C. D. Sherbakoff, O. A. Reinking, and Helen Johann.



FIG. 1.—*Fusarium* bulb rot of young onion plants collected in the field at Walla Walla, Wash., June, 1923: A, withered and yellowed tops, the first field symptom; A, B, and C, dead roots (note progress of the lesion from the scale plate); B, bulb almost completely decayed, a white hyphal mass showing between the scales

other cases incipient infections at harvest continue to advance during storage and transit, finally leaving dry, shrivelled mummies. In the case of red onions it is common for the anthocyan pigment in the outer epidermis to turn green in color for a considerable distance in advance of the actual decay, indicating a change in reaction of the cell sap. *Fusarium* infection of the bulb is often associated with insect wounds, and since this organism is primarily a wound parasite the coincidence of severe epiphytotics of the disease and maggot injury is common.

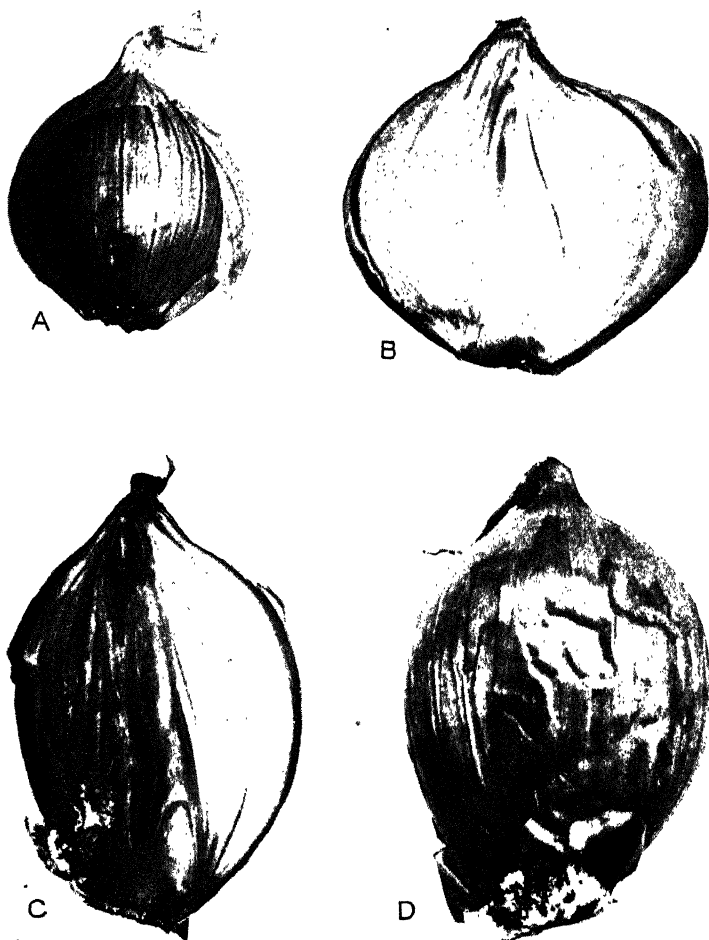


FIG. 2.—*Fusarium* bulb rot following inoculation with a pure culture of *F. zonatum* forma 1. A and B, controls showing wound made by inoculation; C, a section through D, cut so that the wound is visible at the surface with a mass of hyphae. A mealy decay extends from the wound, involving the scale plate and half of the bulb up to the neck. The decayed half is collapsed; D, exterior of an onion showing shriveling and superficial growth of the mycelium at the scale plate as well as at the opening of the wound

Observations of plants in the field made by the writers toward the close of the growing season in California, Colorado, and Washington, and of plants grown and inoculated experimentally in the greenhouse, indicate that this description is generally applicable.

The symptoms of affected bulbs vary with the moisture content of the bulb and of the air. So far there seems to be no difference in symptoms correlated with different species of *Fusarium*, as in the

tuber rots of the Irish potato. Freshly infected bulbs show nearly vinaceous buff to avellaneous⁴ mealy decay extending upward from the scale plate, which in a humid atmosphere usually becomes covered with a white to pinkish cottony mycelium. (Fig. 2, D.) If the bulb at this stage is exposed to dry air, it loses water rapidly, the scales become tough and leathery, and the bulb becomes a mummy. However, if the bulb is kept under moist conditions the scales become filled with mycelium, which forms rows of cushions extending along the veins, and these are visible through the epidermis. (Fig. 2, C.) If the bulb reaches this stage before drying out, the affected scales, when they do dry out, are powdery and full of spores. There is no foul odor in bulbs affected with *Fusarium* bulb rot. When the bulbs are kept in moist air bacteria often become active, and in conjunction with *Fusarium* species, cause a semiwatery to watery decay which often is foul smelling. This decay, due to bacteria and *Fusarium* species, is not, however, as wet or slimy as that caused by bacteria alone.

It should be pointed out that *Fusarium* species, which upon inoculation into a bulb do not produce a decay by themselves, when associated with bacteria progress through the scales of the bulb, producing the plectenchymatic cushions and spore masses present in bulbs attacked by truly pathogenic *Fusaria*. The decay so produced differs from true *Fusarium* decay only in that it is semiwatery instead of mealy and has a foul odor, but can not be distinguished from that caused by a pathogenic species plus bacteria. This accounts for the fact that many bulbs naturally infected yield only nonpathogenic *Fusaria* when they are cultured.

PROOF OF PATHOGENICITY

To demonstrate the pathogenicity of the organisms isolated from decaying onions, inoculation experiments were conducted with growing plants and with mature bulbs.

INOCULATION OF SETS

Sets of red, yellow, and white onions were selected for freedom from disease. They were placed for 15 minutes in 1:1,000 corrosive sublimate solution and then rinsed in sterile distilled water. They were planted in soil which had been autoclaved at 15 pounds for two hours. Eight-inch pots were used and five bulbs were planted in each. Two of the bulbs of each pot were wounded by cutting a small piece out of the side of the scale plates (1 mm. wide, 4 to 5 mm. long, and 3 mm. deep). The bulbs were placed on pieces of *Melilotus* stems inoculated with the culture under test.

There was no stunting or evidence of disease until the plants were approaching maturity. (Fig. 3.) Plants grown in inoculated soil did not mature faster than the controls. The leaves of both controls and plants in inoculated soil had begun to dry back from the tips and some plants had set seed before the first symptoms of disease developed. (Fig. 4, B.) At this stage plants in the inoculated pots collapsed suddenly with yellowing and wilting. This occurred during a period of exceedingly hot weather following a sudden rise of temperature (fig. 5), and is in harmony with the findings of Walker and Tims (12, p. 693). Examination of the bulbs of such collapsed

⁴ Designations of color are made according to Ridgway (7).

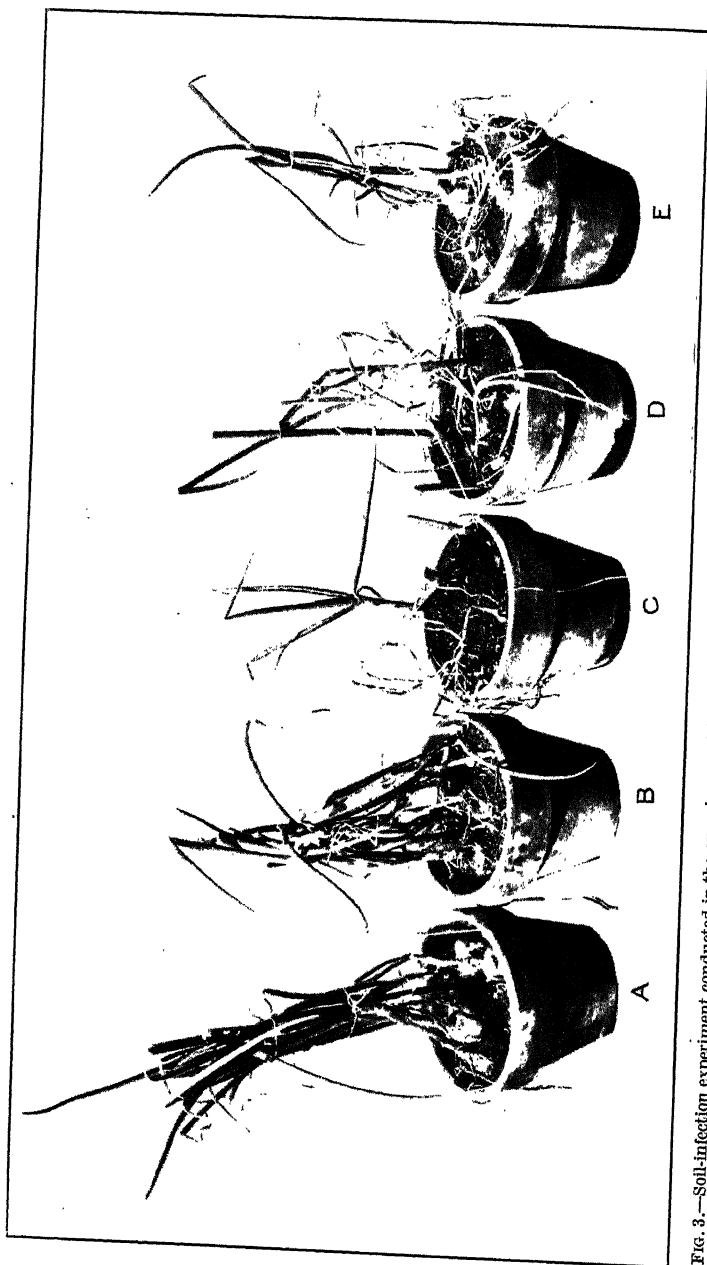


FIG. 3.—Soil-infection experiment conducted in the greenhouse with various species of *Fusarium*. Age of plants, 15 weeks. A, inoculated with a strain of *F. moniliforme* (1624-57); B, control; C, inoculated with *F. zaeforme* forma 1 (1490); D, with *F. zaeforme* forma 2 (1454-6); and E, inoculated with a strain of *F. cepae* Hanz. (1440-6). All bulbs in A and B were sound and free from *Fusarium* species. Symptoms of disease did not become apparent in the other plants until they had begun to mature. All the bulbs were examined and cultured. The bulbs of dead and dying plants in C, D, and E were decayed, and the inoculated *Fusarium* species were recovered from them in culture.

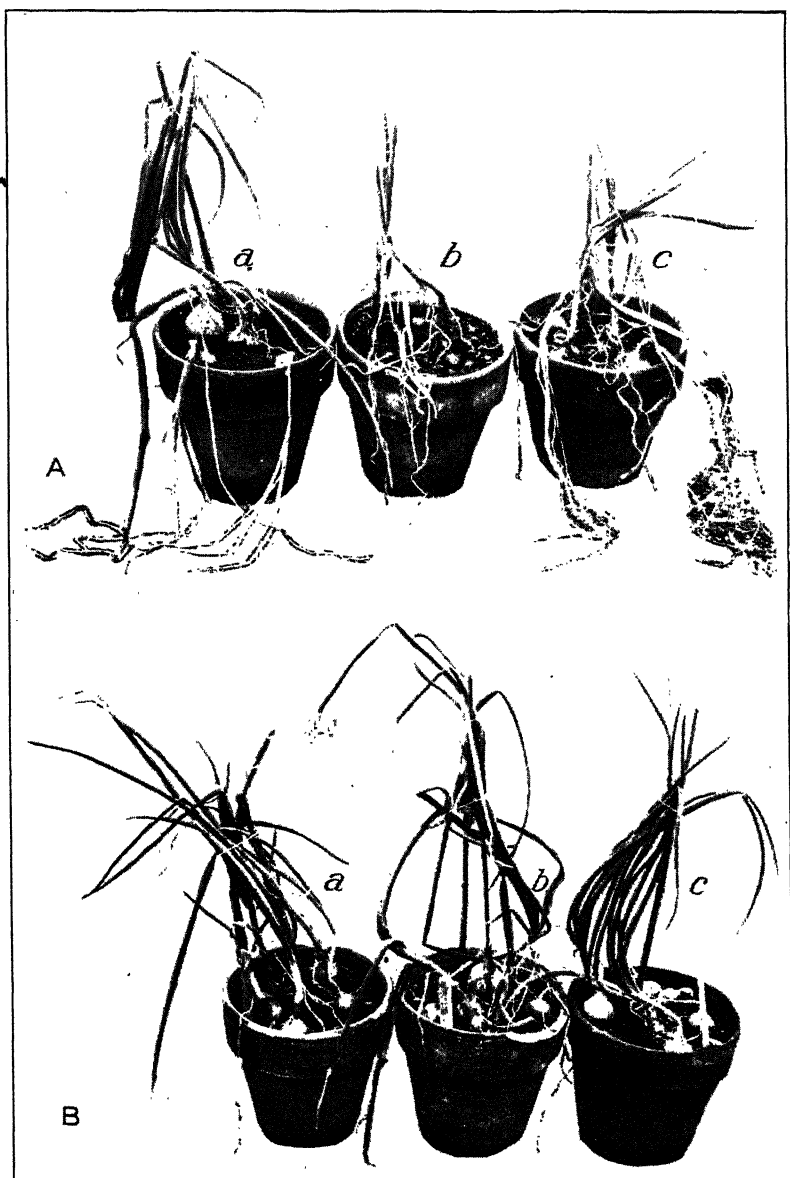


FIG. 4.—Soil inoculation experiments with red, white, and yellow varieties of onions. A, soil inoculated with *Fusarium zonatum* forma 1: a, white variety; b, red; c, yellow. B, soil inoculated with *F. mali*: a, red variety; b, yellow; c, white. Age of plants in A and B, 15 weeks. Death of tops in A was due to age and bulb decay; in B to age alone

plants revealed typical bulb rot and the inoculated organisms were recovered by culture. (Table 1.) In some cases the rot was associated with maggot injury in the nonwounded bulbs. The bulbs of the controls were examined critically to determine whether the dying-back noted in the leaves could be associated with any abnormal condition of the bulbs. Out of 45 controls only one bulb showed decay. It had been injured by maggots and mites, and bacteria and a *Fusarium* belonging to the section *Martiella*, none of which were found to be pathogenic to onions, developed in culture.

Fusarium mali (fig. 4, B), *F. vasinfectum*, *F. oxysporum*, *F. martii* var. *minus*, *F. bulbigenum*, and *F. moniliforme* (fig. 3, A), all isolated from decayed bulbs, were used in this experiment, but did not produce

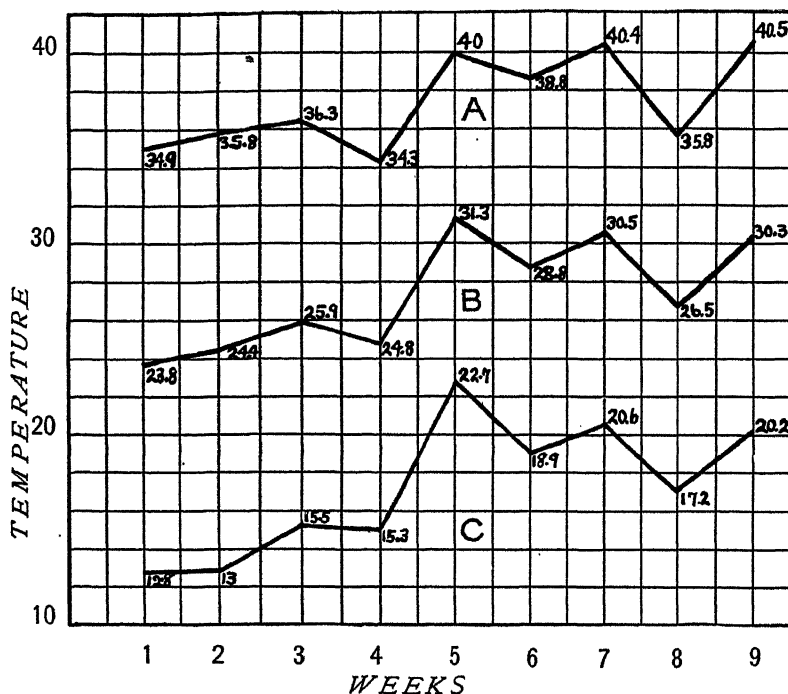


FIG. 5.—Graph showing average maximum, mean, and minimum air temperatures prevailing during the last nine weeks (May 5 to June 30) of soil inoculation experiment begun March 10, 1925. The first symptoms of the disease did not appear until June 12, immediately following the onset of high temperatures. A, maximum (average); B, mean (average); C, minimum (average). Temperature in degrees centigrade

decay. In bulb inoculations these also proved nonpathogenic with the exception of *F. moniliforme*, which sometimes produced rot.

Some of the onions from inoculated soil, which showed no bulb rot at the conclusion of the experiment in the greenhouse, were held to determine whether they would develop bulb rot in storage. They were stored in clean paper bags at room temperature (20 to 24° C.) for several months. At the end of this period some of these bulbs had developed typical bulb rot. (See Table 2.) The lesions were cultured and the inoculated organism was recovered. These experimental facts show that bulbs, apparently free from decay and wounds, harvested from infested soil can develop the disease in transit and storage.

TABLE 1.—*Inoculation of sets*

Culture	Red				Yellow				White			
	Wounded		Unwounded		Wounded		Unwounded		Wounded		Unwounded	
	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed
1456-c (F. zonatum forma 2).....	4	1	6	2	4	1	6	^a 3	4	1	6	0
1490 (F. zonatum forma 1).....	4	4	6	0	4	2	6	3	4	1	6	1
1440-c (F. cepae).....	4	1	6	0	4	^b 2	6	1	4	2	6	1
1660 (F. mali).....	4	0	6	0	4	0	6	0	4	0	6	0
1324 (F. vasinfectum).....	4	0	6	0	4	0	6	0	4	0	6	0
1626-b (F. oxysporum).....	4	0	6	0	4	0	6	0	4	0	6	0
1495 (F. martii var. minus).....	4	0	6	0	4	^c 2	6	0	4	0	6	^c 1
1624-b (F. moniliforme).....	4	0	6	0	4	0	6	0	^d 1	6	0	0
1407 (section Martiella).....	4	0	6	0	4	0	6	0	4	0	6	0
1455-d (F. bulbigenum).....	4	0	6	^e 1	4	^e 1	6	0	4	0	6	0
Control.....	6	0	9	0	6	^f 0	9	0	6	^f 1	9	0

^a 1 bacteria and maggots.^b 1 plus maggots.^c Bulb injured by sow bugs; rot plus bacteria.^d Slight.^e Rot plus *Penicillium*.^f Bacteria and Martiella group *Fusarium* plus maggots and mites.TABLE 2.—*Greenhouse onions kept in storage*

Culture No.	Onion variety	Wounded or unwounded	Number of bulbs stored	Number of bulbs showing decay
1624-b' (F. moniliforme).....	White.....	Wounded.....	2	0
	Yellow.....	do.....	2	0
1660 (F. mali).....	Yellow.....	Unwounded.....	1	0
	White.....	do.....	1	0
1455-d (F. bulbigenum).....	Yellow.....	Unwounded.....	2	0
	Red.....	Wounded.....	1	0
	White.....	do.....	1	0
	do.....	Unwounded.....	2	0
1626-b (F. oxysporum).....	Yellow.....	Wounded.....	2	0
	do.....	Unwounded.....	3	0
	Red.....	Wounded.....	1	0
	do.....	Unwounded.....	1	0
1407 (section Martiella).....	Yellow.....	Unwounded.....	1	0
	do.....	Wounded.....	1	(^a)
	White.....	do.....	1	0
	do.....	Unwounded.....	1	0
1456-c (F. zonatum forma 2).....	Red.....	Wounded.....	1	(^a)
	Yellow.....	Unwounded.....	1	0
	White.....	do.....	2	1
	do.....	Unwounded.....	1	0
1495 (F. martii minus).....	White.....	Unwounded.....	1	(^b)
	Red.....	do.....	1	1
1490 (F. zonatum forma 1).....	Yellow.....	Wounded.....	1	1
	White.....	do.....	1	1
1440-c (F. cepae).....	Red.....	Unwounded.....	2	1
	White.....	do.....	1	1

^a Rot plus *Penicillium*.^b *Penicillium* only.

BULB INOCULATIONS

For these experiments disease-free bulbs were selected of white, yellow, and red varieties. The bulbs were washed, immersed in 1:1,000 mercuric chloride for one hour and then rinsed in sterile

water. Early tests with wounded and nonwounded bulbs indicated that the *Fusarium* species pathogenic to onion bulbs are wound parasites. (See Table 3.) For this reason in all later pathogenicity tests, a stab was made with a sterile probe just above and extending into the scale plate. (Fig. 2, A, B.) The inoculum consisted of spores taken from 10-day-old potato dextrose tubes and was inserted in the stab with a needle. The bulbs were then placed in sterile moist chambers and incubated at 25° C. for 48 hours. They were next put into clean new paper bags (a bag for each species of *Fusarium*) and stored in a cabinet at room temperature (20 to 24° C.) for periods of two weeks to four months. Adequate controls were run.

TABLE 3.—Summary of inoculation of wounded and unwounded bulbs

[Single-spore cultures were used for inoculations]

Culture No.	Locality from which onion was obtained	Region from which isolation was made	Inoculations	
			Wounded	Unwounded
299.....	Spain.....	Bulb.....	+	—
404.....	Texas.....	Bulb (side lesion).....	—	—
434-a.....	Colorado.....	Bulb.....	—	—
434-b.....	do.....	do.....	+	—
455.....	do.....	do.....	+	—
456.....	do.....	do.....	+	—
456.....	do.....	do.....	+	—
457.....	do.....	do.....	+	—
458.....	do.....	do.....	+	—
458.....	do.....	do.....	+	—
490.....	do.....	do.....	+	—
491-a.....	do.....	do.....	—	—
491-b.....	do.....	do.....	—	—
524-a.....	do.....	do.....	+	Rhizopus.
524-b.....	do.....	do.....	Penicillium.....	—
524-c.....	do.....	do.....	+	—
524-d.....	do.....	do.....	+	—
592.....	Spain.....	do.....	—	—
592-a.....	do.....	Same bulb as 592.....	—	—
592-b (a).....	do.....	Bulb.....	—	—
592-b (b).....	do.....	Same bulb.....	+	—
602.....	Illinois.....	Onion set.....	—	—
673.....	Colorado.....	Bulb.....	—	Rhizopus.
840-a.....	Texas.....	do.....	+	—
840-b.....	do.....	do.....	+	—
1182.....	Spain.....	do.....	+	—
1260-b.....	Colorado.....	do.....	+	—
1263.....	do.....	do.....	+	—
1264.....	do.....	do.....	—	—
1322.....	do.....	do.....	+	—
1323.....	do.....	do.....	+	—
1324.....	do.....	do.....	—	—

Generally mycelium developed in the mouth of the wounds in 48 hours, even in those onions which did not become diseased. In those which rotted a depressed area developed about the mouth of the wound in 4 to 5 days, depending upon the temperature. The sunken area increased but not commensurately with the volume of the affected tissues. (Fig. 2, D.) Affected tissues appeared plump, but when pressure was applied were found to be spongy instead of firm.

There were very few contaminations and these were due to bacteria and to *Penicillium* spp. (Table 4.) The latter did not markedly affect the nature of the decay but bacteria did, changing the decay from a mealy to a watery one.

TABLE 4.—*Summary of inoculation experiments of wounded bulbs*

[Inoculations made with single-spore cultures]

Culture No.	Locality from which bulb was obtained	Region of bulb from which isolation was made	Group to which culture belongs	Number of inoculations	Number of takes
1490	D*	B ^b	Elegans (zonatum forma 1)	28	28.
1491	D	B	do.	14	14.
1459-b	W	B	do.	15	15.
1451-c	W	(B)	do.	16	16.
1430-a	W	B	do.	14	14.
1440-c	W	(B)	Elegans (cepae emend.)	28	25.
1456-c	W	(B)	Elegans (zonatum forma 2)	28	25.
1458-c	W	B	do.	14	14.
1443-b	W	B	do.	14	11.
1475-d	W	B	do.	14	14.
1454-b	W	B	do.	16	16.
1484-a	W	(B)	Elegans	17	14.
1455-d	W	B	Elegans (bulbigenum)	30	4, slight; 6, rot plus bact. ^c
1426-B	W	B	Elegans	14	0.
1450	D	B	do.	15	0.
1496-x	D	B	do.	14	0.
1493	D	B	do.	14	3?
1440-ax	W	(B)	do.	14	3?
1433-b	W	(B)	do.	14	3; 1, rot plus bact.
1475-b	W	B	do.	14	2, rot plus bact.
1471-b	W	(B)	do.	14	0.
1429-c	W	(B)	do.	14	1.
1457-d	W	(B)	do.	14	0.
1446-e	W	B	do.	14	0.
1409	S	B	do.	14	0.
1322	D	B	Liseola (F. moniliforme)	10	8.
840-b	T	B	do.	12	10.
1624-b'	W	Stem plate	do.	10	2; 2, rot plus bact.
1324	D	Side lesion of bulb	Elegans (F. vasinfectum)	16	3, plus bact; 4, rot plus bact.
1495	D	B	Martiella (F. martii minus)	19	2, slight.
1407	S	R	Martiella	15	0.
1626-b	W	B	Elegans (F. oxysporum)	16	1, slight; 1, rot plus bact.
1475-c	W	R	Elegans	14	0.
486	D	B	Martiella	14	5, slight; 5, rot plus bact.
1264	D	B	Discolor	12	1, rot plus bact; 1, rot plus Penicillium.
592	Spain	B	Elegans	11	5, slight.
299	Spain	B	do.	10	1, slight; 4, rot plus bact.
1397	S	Stem plate	do.	6	3, slight.
1401	S	R	do.	6	0.
1404-a	S	R	do.	6	0.
1404-B	S	R	do.	6	0.
1405	S	R	Martiella	6	0.
1406-a	S	R	do.	6	0.
1406-B	S	R	Elegans	6	2 (1 slight).
1426-A	W	B	do.	6	5.
1427-A	W	(B)	do.	6	6.
1427-B	W	(B)	do.	6	6.
1429-A	W	(B)	do.	6	5; 1, Penicillium.
1430-c	W	(B)	do.	6	6.
1431-d	W	(R)	do.	6	5.
1432-a	W	B	do.	6	5; 1 Penicillium.
1432-c	W	R	do.	6	0.
1432-E	W	Bulb of 1432-c	do.	6	6.
1433-d	W	B	do.	6	2; 1 rot plus bact.
1434-B	W	(B)	do.	6	5; 1 contaminated.
1434-d	W	(B)	do.	6	6.
1435-b	W	Stem plate	do.	6	6.
1435-d	W	(B)	do.	6	3.
1436-a	W	B	do.	6	5; 1 contaminated.
1436-c	W	Root of 1436-a	Martiella	6	0.
1437-b	W	B	Elegans	6	5.
1437-d	W	B	do.	6	5.
1438-a	W	B	do.	6	6.
1438-c	W	B	do.	6	6.
1439-b	W	B	do.	6	2, slight; 1, rot plus bact.

* D=Delta, Colo.; S=Stockton, Calif.; T=Texas; W=Walla Walla, Wash.

^b B=bulb with roots intact; (B)=bulb with roots entirely destroyed by decay; R=root from rotted bulb; (R)=pink roots from bulbs without bulb rot.^c "Plus bact."=bacteria present in addition to Fusarium.

TABLE 4.—Summary of inoculation experiments of wounded bulbs—Continued

Culture No.	Locality from which bulb was obtained	Region of bulb from which isolation was made	Group to which culture belongs	Number of inoculations	Number of takes
1439-c.	W	Stem plate	Elegans	6	6.
1440-a.	W	(B)	do.	6	6.
1441-a.	W	(R)	Martiella	6	0.
1442-a.	W	(B)	do.	6	2, slight.
1442-c.	W	(B)	Elegans	6	6.
1443-d.	W	B	do.	6	6.
1445-b.	W	(B)	do.	6	5.
1445-d.	W	(B)	do.	6	5; 1, contaminated.
1446-b.	W	(B)	do.	6	6.
1447-a.	D	(B)	do.	6	5.
1447-c.	D	(B)	Martiella	6	0.
1448-a.	D	(B)	Elegans	6	6.
1449-c.	D	(B)	do.	6	6.
1451-b.	W	(B)	do.	6	4.
1452-b.	W	B	do.	6	6.
1452-c.	W	B	do.	6	4; 2, contaminated.
1456-a.	W	(B)	Martiella	6	1, rot plus bact.
1457-a.	W	B	Elegans	6	5.
1457-ax	W	(B)	do.	6	6.
1458-d.	W	B	do.	6	4.
1460-d.	W	(B)	do.	6	5.
1461-a.	W	B	do.	6	5.
1472-a.	W	B	do.	6	4.
1472-ax	W	B	do.	6	6.
1473-a.	W	(B)	do.	6	4.
1473-d.	W	(B)	do.	6	6.
1474-b.	W	B	do.	6	4.
1475-a.	W	R	do.	6	5.
1488.	D	B	do.	6	6.
1492.	D	B	do.	8	2; 2, rot plus bact
1494.	D	B	do.	6	2; 2, rot plus bact
1494-x.	D	B	do.	6	6.
1496-a.	D	B	Martiella	6	3 (2 slights).
1496-b.	D	B	Elegans	6	4 (2 con.).

It should be noted that with but one exception (1475-a, which belongs to section *Elegans*) strains isolated from the roots did not produce bulb rot. Additional work with other cultures not listed in the table indicates that, with few exceptions, the organisms occurring on roots (10) are not pathogenic to bulbs. It is significant in this connection that inoculation of bulbs with *F. mali*, Taub., which is reported to be the cause of pink root (11), proved negative (Tables 1 and 6.)

VARIETAL SUSCEPTIBILITY

No difference in susceptibility in red, white, and yellow onion varieties was noted, as indicated by Tables 1 and 5 and Figure 4.

TABLE 5.—Summary of inoculations of different varieties of onions

Culture	Red		Yellow		White	
	Number tested	Number decayed	Number tested	Number decayed	Number tested	Number decayed
1496-a (section Martiella)	1	0	1	0	1	0
1404-a (section Elegans)	1	0	1	0	1	0
1490 (<i>F. zonatum</i> forma 1)	3	(^a)	2	2	2	2
1456-c (<i>F. zonatum</i> forma 2)	3	3	2	2	2	2
1626-b (<i>F. oxysporum</i>)	3	0	2	0	2	0
1440-c (<i>F. cepae</i> emend)	3	2	2	2	2	(^b)
1455-d (<i>F. bulbigenum</i>)	3	0	2	0	2	(^c)
1495 (<i>F. martii</i> var. minus)	3	0	2	0	2	0
1624-b' (<i>F. moniliforme</i>)			2	0	2	0
1324 (<i>F. vasinfectum</i>)			2	0	2	0
1407 (section Martiella)			2	0	2	0
Control	2	0	3	0	2	0

^a 3 (1 slight).^b 2 (1 slight).^c 1? (in dead tissue).

IDENTITY OF THE PATHOGENES

A study of cultures shows that with the exception of 840-b, 1624-b', and 1322, which belong to section *Liseola*, all of the pathogenic forms fall into section *Elegans* (13). It also shows, however, that by no means all species of *Elegans* isolated from onions are pathogenic.

In their early grouping the writers found a considerable number of organisms which developed an abundant pionnotes. Of these cultures 1490 and 1491 from Delta, Colo., and 1459-b, 1451-c, and 1430-a from Walla Walla, Wash., were found to be identical. Culture 1490 was selected for study in determining this species. It was identified by the writers as *F. zonatum* (Sherb.) Wollenweber (15). It was also compared with a culture furnished by Walker and Tims which they had described as *F. cepae* (Hanzawa) emend. Walker and Tims (12). The writers' culture 1490 seemed identical not only with *F. zonatum* but also with *F. cepae* furnished them by Walker and Tims. At the Fusarium conference it was decided that the organism described by Walker and Tims as *F. cepae* emend. Walker et Tims (12) is *F. zonatum* forma 1 and that 1490 is identical with it.

The description of 1490 follows.

STRAIN 1490—FUSARIUM ZONATUM (SHERB.) WR. FORMA 1

Mycelium typically scant, when present fine fluffy white to grayish white.

Substratum typically light buff to pale ochraceous buff, rarely on very acid media in dim light from vinaceous fawn to russet vinaceous (the deep colors described by Walker and Tims (12, p. 684) for their strain were never obtained with 1490); no sclerotia but small warm sepia to warm blackish brown plectenchymatic cushions on steamed rice; color on rice from cream white to salmon buff or salmon color; slight lilac odor on rice; chlamydospores (fig 6, B) abundant in spores and mycelium, terminal and intercalary, solitary, in chains, and in clusters, smooth to echinate; microconidia (fig. 6, A) usually not numerous, scattered in aerial mycelium or present in sporodochia or pionnotes, ellipsoidal to ovoid or slightly disovisentral, mostly 0-septate 7.8 by 2.7 μ average (5 to 11.6 by 1.6 to 4.15 μ), some 1-septate 13.2 by 2.7 μ average (8 to 18.2 by 1.6 to 3.32 μ); macrospores (fig. 6, A and B) sickle-shaped, pedicellate, slightly constricted at apex, borne on irregularly branched sporophores, scattered or in sporodochia and pionnotes; pionnotes typically present, salmon buff to ochraceous salmon; macroconidia 3 to 5-septate, predominately 3-septate 37.1 by 3.8 μ average (27.2 to 46.5 by 2.8 to 4.56 μ); 4-septate 41.5 by 3.92 μ average (34.4 to 50 by 3.2 to 4.56 μ); 5-septate 43.4 by 4 μ average (37 to 49.8 by 3.32 to 4.56 μ).

Zonation marked on some media.

Habitat: On decaying bulbs of onion (*Allium cepa* L.), Delta, Colorado.

Measurements⁵ of spores on different media⁶ are as follows (numbers in parentheses after averages indicate number of spores measured. At least five fields were counted for percentages):

On potato-dextrose-agar⁷ tube, culture 35 days old, conidia from pionnotes:

5-septate, 8 per cent, 41.8 by 4.2 μ average (5), 37 to 45 by 4 to 4.5 μ .

4-septate, 38 per cent, 41.4 by 4.1 μ average (10), 39 to 43 by 4 to 4.5 μ .

3-septate, 54 per cent, 38.7 by 4.1 μ average (10), 37 to 41 by 4 to 4.5 μ .

On oatmeal-agar tube, culture 26 days old, conidia from pionnotes:

5-septate, 2.8 per cent, 45.5 by 4 μ average (5), 43 to 48 by 3.32 to 4.15 μ .

4-septate, 6.7 per cent, 43.5 by 4.1 μ average (10), 38.2 to 50 by 3.32 to 4.5 μ .

3-septate, 49.6 per cent, 41.5 by 4 μ average (15), 34 to 45 by 3.32 to 4.5 μ .

2-septate, 2.5 per cent.

1-septate, 1.5 per cent, 14.3 by 2.9 μ average (3), 10 to 18.2 by 2.5 to 3.32 μ .

0-septate, 36.9 per cent, 8.8 by 2.7 μ average (10), 5 to 11.6 by 1.7 to 3.32 μ .

⁵ Only normal spores were measured. For criteria of the norm see (9, 14, 17).

⁶ The formulae followed for making media are those used at the Fusarium conference (17) or those given by Sherbakoff (9).

⁷ All potato-dextrose agar, unless otherwise stated, is 2 per cent dextrose and 2 per cent agar.

On oatmeal-agar plate, culture 36 days old, conidia from pionnotes:

5-septate, 1.5 per cent.

4-septate, 15.4 per cent, 38.4 by 3.4μ average (5), 34.4 to 40 by 3.2 to 3.6μ .

3-septate, 80.2 per cent, 36 by 3.4μ , average (11), 27.2 to 43.2 by 2.8 to 3.6μ .

0-septate, 2.9 per cent.

Chlamydospores, 6.5 by 6.7μ average (4), 5.6 to 8 by 4.8 to 8μ .

On potato-tuber plug, culture 18 days old, conidia from sporodochium:

5-septate, 0.5 per cent, 38.3 by 3.7μ average (4), 37 to 38.8 by 3.7μ .

4-septate, 3.7 per cent, 36.5 by 3.7μ average (8), 35 to 38.8 by 3.7μ .

3-septate, 87.5 per cent, 33.3 by 3.6μ average (22), 27.8 to 40.7 by 1.9 to 3.7μ .

2-septate, 0.3 per cent.

1-septate, 6.7 per cent.

0-septate, 1.3 per cent.

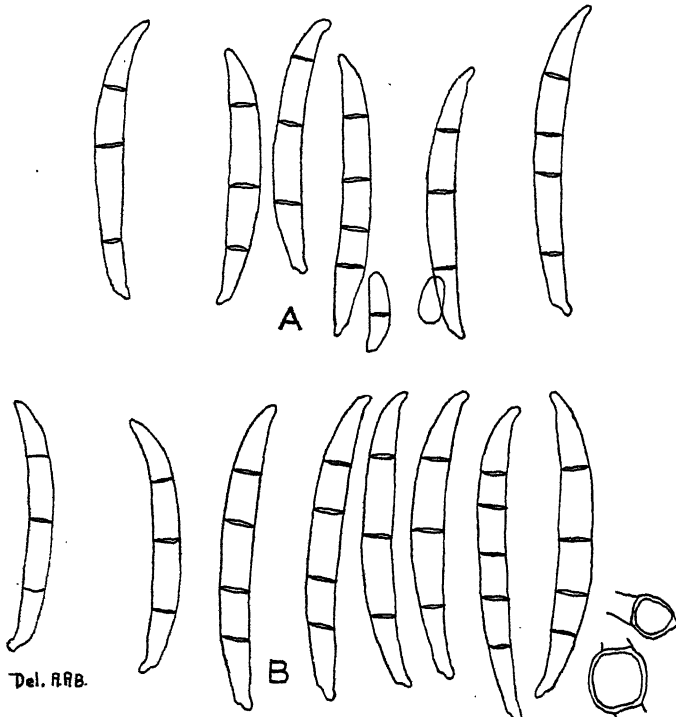


FIG. 6.—Spores of 1490, *Fusarium zonatum* (Sherb.) Wr. forma 1: A, spores from sporodochium on 35-day-old rice culture; B, macrospores and chlamydospores from pionnotes on 35-day-old potato-agar tube culture. Camera lucida drawings. All $\times 1,000$

On potato-tuber plug, culture 37 days old, conidia from sporodochium:

5-septate, 1.2 per cent, 45 by 3.7μ average (2), 44 to 46.5 by 3.32 to 4.15μ .

4-septate, 23 per cent, 42.7 by 3.9μ average (10), 36.5 to 46.5 by 3.32 to 4.15μ .

3-septate, 73.2 per cent, 36.5 by 4μ average (20), 30 to 46.5 by 3.32 to 4.15μ .

1-septate, 1.2 per cent, 12.9 by 2.9μ average (7), 10 to 15.8 by 2.5 to 3.32μ .

0-septate, 1.4 per cent, 7 by 2.9μ average (10), 5 to 8.3 by 2.5 to 4.15μ .

On Melilotus stems, culture 26 days old, conidia from pionnotes:

4-septate, 1.5 per cent, 38.4 by 3.8μ average (2), 36.8 to 40 by 3.6 to 4μ .

3-septate, 64.5 per cent, 32.5 by 3.5μ average (10), 29 to 36 by 3.2 to 4μ .

1-septate, 1.5 per cent, 12.8 by 2μ average (4), 8 to 16 by 1.6 to 2μ .

0-septate, 32.5 per cent, 8.5 by 2.1μ average (3), 8 to 9.6 by 1.6 to 2.8μ .

On onion-agar tubes, culture 21 days old, conidia from pionnotes:

6-septate, 0.6 per cent.

5-septate, 1.5 per cent, 45.5 by 4.2μ average (7), 41.5 to 49.8 by 4.15 to 4.56μ .

4-septate, 10.6 per cent, 43.5 by 4μ average (15), 40 to 48 by 3.32 to 4.56μ .

3-septate, 34 per cent, 41.6 by 4μ average (20), 38.2 to 46.5 by 3.32 to 4.5μ .

Small 3-septate, 34 per cent, 27.5 by 3.5μ average (7), 25 to 31.5 by 2.9 to 4.15μ .

2-septate, 0.2 per cent, 16.6 by 3.32μ average (1).

1-septate, 2.4 per cent, 13.3 by 2.9μ average (5), 11.6 to 15 by 2.5 to 3.32μ .

0-septate, 50.7 per cent, 7.6 by 2.8μ average (11), 5 to 10 by 1.7 to 4.15μ .

On steamed rice, culture 35 days old, conidia from old sporodochium:

5-septate, 1 per cent.

4-septate, 12 per cent, 40.5 by 4μ average (2), 38 to 43 by 4μ .

3-septate, 67 per cent, 35.6 by 3.82μ average (10), 33 to 38 by 3.5 to 4μ (30 by 3.5μ).

2-septate, 1 per cent.

1-septate, 7 per cent.

0-septate, 12 per cent.

On 20 per cent dextrose-agar plate, culture 40 days old, conidia from pionnotes:

4-septate, 2.9 per cent.

3-septate, 12.8 per cent.

2-septate, 3.1 per cent.

1-septate, 0.7 per cent.

0-septate, 80.5 per cent.

Average of all media:

5-septate, 43.4 by 4μ average (23), 37 to 49.8 by 3.32 to 4.56μ .

4-septate, 41.5 by 3.92μ average (62), 34.4 to 50 by 3.2 to 4.56μ .

3-septate, 37.1 by 3.8μ average (118), 27.2 to 46.5 by 2.8 to 4.56μ .

Small 3-septate, 27.5 by 3.5μ average (7), 25 to 31.5 by 2.9 to 4.15μ .

2-septate, 16.6 by 3.32μ average (1).

1-septate, 13.2 by 2.7μ average (19), 8 to 18.2 by 1.6 to 3.32μ .

0-septate, 7.8 by 2.7μ average (34), 5 to 11.6 by 1.6 to 4.15μ .

Chlamydosporos, 6.5 by 6.7μ average (4), 5.6 to 8 by 4.8 to 8μ .

One culture of the pionnotes group, 1440-c (from Walla Walla), which is pathogenic, differed from all the others. It seemed to be close to *Fusarium sclerotioides* Sherb. and *F. aurantiacum* (Link, Sacc.). The description of *F. sclerotioides* more fully fits the organism because of the reference to hyphal knots and wartlike plectenchyma bodies (9, p. 215). At the conference mentioned above it was agreed that *F. sclerotioides* was *F. aurantiacum*, but that the writers' 1440-c differed so much from *F. aurantiacum* that it should be considered a different species. In view of the fact that the name *F. cepae* had been used and the meager description given by Hanzawa (5) was not at variance with that of 1440-c, except for the greater diameter of spores (4.5 to 6.3μ given by Hanzawa), it was decided that 1440-c should be described as *F. cepae* Hanzawa emend. Hanzawa's figures show abnormal, swollen conidia, and it is likely that he used these for his measurements. Wollenweber in his discussion of *Fusarium* in Sorauer's "Handbuch" (16, p. 173) has called attention to this possibility.

The morphological and cultural characters of the organism have been determined on various media and are given below.

FUSARIUM CEPAE HANZ. EMENDED

Fine, fluffy white aerial mycelium, sometimes Botrytis green at base of slant or on agar rich in dextrose; Artemisia green plectenchyma on potato-tuber plugs; substratum usually colorless but on agar very rich in dextrose (20 per cent) dark vinaceous purple; color on steamed rice salmon buff to light salmon orange; sclerotia on potato-tuber plugs and agar, one-half millimeter in diam-

eter, dark dull yellowgreen to duck green; loose hyphal knots to irregular plectenchymatic bodies 1 to 2 millimeters in diameter, white to pale salmon buff, sometimes present in plate cultures. These may bear sporodochia; Chlamydospores (fig. 7, A, c, d, e, and E, b) abundant in mycelium and conidia, terminal and intercalary, unicellular (6.4 by 6.4 μ) to two celled, solitary, in chains, or in groups, smooth to echinate; microconidia (fig. 7, A a, B, E, a), borne scattered and in false heads on the aerial mycelium, also present in sporodochia and pionnotes, ellipsoidal to ovoid or slightly dorsiventral, mostly unicellular, 6 to 9 (13) by 2 to 3.25 μ (7.5 by 2.7 μ average), rarely 1-septate, 11.5 to 17 (20) by 2 to 3 μ (12.7 by 2.75 μ average); Aurantiacumlike macroconidia (fig. 7, A, C, D, E) borne on irregularly branched conidiophores, scattered or in sporodochia or pionnotes, light vinaceous cinnamon to ochraceous salmon, sickle-shaped, pedicellate, slightly constricted at apex, 3 to 5 septate (rarely 2 to 6), predominately 3-septate 25 to 46 by 3 to 4.5 μ (35.7 by 3.8 μ average), on some media producing in addition small 3-septate spores, 22.5 to 28 by 2 to 3 μ (25.7 by 2.7 μ average); 2-septate 16.5 to 17.6 by 2 to 3 μ (17.4 by 2.8 μ average); 4-septate, 32 to 51 by 3 to 5 μ (40.9 by 3.7 μ average); 5-septate, 38 to 53 by 2.7 to 5 μ (44.4 by 3.9 μ average); 6-septate, 46.8 to 55 by 2.5 to 3.7 μ (52.4 by 3.3 μ average).

Differs from *F. aurantiacum* by the abundance of the chlamydospores in mycelium and conidia, by absence of red color on rice, and presence of slight lilac odor on steamed rice.

Habitat: On decaying bulbs of onion (*Allium cepa* L.) Walla Walla, Washington.

LATIN DESCRIPTION

Aerio mycelio subtile, ex albo, interdum "Botrytis green" ad radicem tubi agaris vel in *Solani tuberosi* agare perglucoso; stromate plectenchymico "Artemisia green" in tuberibus *Solani tuberosi*; substrato plerumque hyalino, sed "dark vinaceous purple" in *Solani tuberosi* perglucoso (20%); colore in *Oryza cocta* "salmon buff" vel "light salmon orange"; sclerotiis in tuberibus et agare *Solani tuberosi* (1-2 mm. diam.) "dark dull yellow green" vel "duck green"; laxis nodis in hyphalibus apicibus vel enormibus plectenchymis corporibus (1-2 mm. diam.), albido vel "pale salmon buff," interdum in culturis in Petri patina, interdum sporodochia ferentibus; chlamydosporis (fig. 7, A, c, d, e, and E, b) largis in mycelio et conidiis, terminalibus vel intercalariis, continuis (6.4 \times 6.4 μ), 1-septatis, singulis, catenulatis, acervalibus, levibus vel rugulosis; microconidiis (fig. 7, A, a, B, E, a), aerio mycelio instratis vel capitulis falsis dispositis, quoque in sporodochiis vel in pionnote, ellipsoideo-ovoideis vel vix dorsiventralibus, plerumque continuis 6 to 9 (13) by 2 to 3.25 μ (7.5 by 2.7 μ average); rarius 1-septatis, 11.5 to 17 (20) by 2 to 3 μ (12.7 to 2.75 μ average); macroconidiis (fig. 7, A, C, D, E) similibus *F. aurantiaco* ad conidiophoros irregulariter ramosos dispositis, aerio mycelio instratis, vel in sporodochiis et in pionnote, "light vinaceous cinnamon" vel "ochraceous salmon," falcatis, pedicellatis, apice leviter constricto, 3 to 5 septate (raro 2 to 6 septate); plerumque 3 septate 25 to 46 by 3 to 4.5 μ (35.7 by 3.8 μ average); in aliquis mediis quoque minoribus 3 septate conidiis 22.5 to 28 by 2 to 3 μ (25.7 by 2.7 μ average); 2 septate 16.5 to 17.6 by 2 to 3 μ (17.4 by 2.8 μ average); 4 septate 32 to 51 by 3 to 5 μ (40.9 by 3.7 μ average); 5 septate 38 to 53 by 2.7 to 5 μ (44.4 by 3.9 μ average); 6 septate, 46.8 to 55 by 2.5 to 3.7 μ (52.4 by 3.3 μ average).

A *F. aurantiaco* (Lk.) Sacc. emend. Wr. differt abundantius chlamydosporiis in mycelio et conidiis, nullo carmineo colore in *Oryza cocta*, et praesentia parvi odoris Syringae in *Oryza cocta*.

Habitat: In bulbis putridis *Allii cepae* L., Walla Walla, Washington.

Measurements of spores on different media are as follows:

On potato-dextrose agar tube, culture 31 days old, conidia from sporodochium: 3-septate, 74 per cent, 33.3 by 4 μ average (10), 31 to 38 by 3.5 to 4.5 μ (28 by 4).

1-septate, 1 per cent, 17 by 2.5 μ average (1).

0-septate, 25 per cent.

On potato-dextrose agar tube, culture 75 days old, conidia from pionnotes:

5-septate, 2 per cent.

4-septate, 12 per cent.

3-septate, 57 per cent.

1-septate, 3 per cent.

0-septate, 26 per cent.

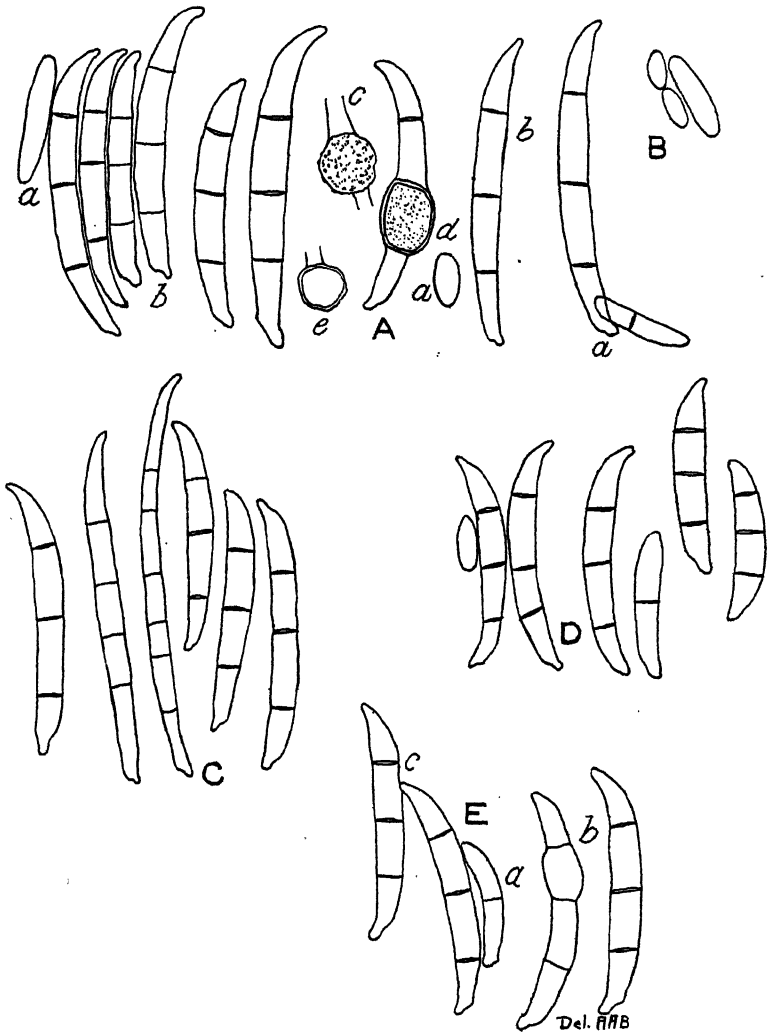


FIG. 7.—Spores of 1440-c, *Fusarium cepae* Hanzawa emended

A.—From pionnotes on oatmeal-agar culture 5 days old: a, microspores; b, macrospores; c, echinate intercalary chlamydospore from mycelium; d, chlamydospore in conidium; e, smooth terminal chlamydospore from mycelium

B.—Microconidia from 32-day-old steamed rice culture

C.—Types of macroconidia from thin submerged pionnotes on 32-day-old potato-tuber plug culture

D.—Conidia from sporodochium (1 mm. diameter) above the mycelium on a 32-day-old *Mellilotus* stem culture

E.—Conidia from sporodochium on potato-agar tube culture 31 days old: a, microspore; b, chlamydospore in macrospore; c, macrospores

Camera lucida drawings. All $\times 1,000$

- On oatmeal-agar tube, culture 26 days old, conidia from sporodochium:
5-septate, 8 per cent, 47.3 by 3.93μ average (6), 46.5 to 49.8 by 3.32 to 4.15μ .
4-septate, 33 per cent, 43.3 by 3.93μ average (10), 39.8 to 51.5 by 3.32 to 4.15μ .
3-septate, 48 per cent, 40.5 by 3.98μ average (10), 36.5 to 43.2 by 3.32 to 4.15μ .
2-septate, 1 per cent, 17.6 by 3.15μ average (3), 16.6 to 18.3 by 2.9 to 3.32μ .
1-septate, 0.5 per cent, 11.5 by 3.00μ average (5), 10 to 13.3 by 2.5 to 3.32μ .
0-septate, 9.5 per cent, 7.5 by 2.65μ average (10), 5 to 11.6 by 1.66 to 3.32μ .
- On oatmeal-agar plate, culture 5 days old, conidia from pionnotes:
5-septate, 6 per cent.
4-septate, 19 per cent.
3-septate, 74 per cent, 38 by 3.75μ average (10), 32.5 to 44 by 3 to 4.5μ .
1-septate, 1 per cent, 14 by 2.75μ (1).
0-septate, 13.5 by 3.25μ average (2), 9 to 18 by 3 to 3.5μ .
- On potato-tuber plug, culture 15 days old, conidia from sporodochium:
7-septate, very rare, 46.8 by 3.9μ (1).
6-septate, very rare, 53.7 by 3.7μ average (2), 51.8 to 55 by 3.7μ .
5-septate, 15 per cent, 41.7 by 2.72μ average (8), 38.8 to 46.8 by 3.7 to 3.9μ .
4-septate, 24 per cent, 40 by 3.8μ average (13), 33.3 to 46.3 by 3.3 to 3.7μ .
3-septate, 59 per cent, 35.8 by 3.8μ average (33), 31.2 to 42.9 by 3.7μ .
0-septate, 2 per cent.
- On potato-tuber plug, culture 32 days old, conidia from thin submerged pionnotes:
6-septate, 2 per cent, 54 by 2.75μ average (2), 53 to 55 by 2.5 to 3μ .
5-septate, 6 per cent, 47.5 by 3.25μ average (2), 47 to 48 by 3 to 3.5μ .
4-septate, 10 per cent, 41 by 3.75μ average (2), 40 to 42 by 3.5 to 4μ .
3-septate, 79 per cent, 33.2 by 3.8μ average (10), 28 to 37 by 3.5 to 4.5μ .
1-septate, 1 per cent.
0-septate, 2 per cent.
- On potato-tuber plug, culture 37 days old, conidia from sporodochium:
5-septate, 10 per cent, 40 by 4.2μ average (10), 38.2 to 53.1 by 4.15 to 4.56μ .
4-septate, 32 per cent, 38.8 by 4.1μ average (10), 34.9 to 43.2 by 3.32 to 4.15μ .
3-septate, 47 per cent, 36.2 by 3.98μ average (10), 32.2 to 38.2 by 3.32 to 4.15μ .
1-septate, 3 per cent, 11.5 by 2.9μ average (5), 8.3 to 14.9 by 2.5 to 3.32μ .
0-septate, 8 per cent, 7 by 2.7μ average (10), 4.2 to 10.8 by 2.5 to 3.32μ .
- On *Meliolus* stems, culture 26 days old, conidia from pionnotes:
Thin pionnotes on drier upper part of the tube: 4-septate, 5 per cent;
3-septate, 83 per cent; 0-septate, 12 per cent.
Thick pionnotes at moist bottom of tube: 4-septate, 1 per cent; 3-septate, 13 per cent; 1-septate, 2 per cent; 0-septate, 84 per cent.
Small 3-septate, 32 by 2.7μ average (10), 22.4 to 35.2 by 2.4 to 3.6μ .
2-septate, 17.6 by 2μ average (2), 17.6 by 2 to 2.4μ .
1-septate, 12.8 by 2μ average (3), 11.2 to 14.4 by 2 to 2.4μ .
0-septate, 9.6 by 2μ average (2), 7.2 to 12.8 by 1.6 to 2μ .
Chlamydospores 6.4 by 6.4μ average (3).
- On *Meliolus* stems, culture 32 days old, conidia from 1 millimeter sporodochium above the mycelium:
3-septate, 24 per cent, 27.9 by 4.1μ average (10), 25 to 32 by 3.5 to 4.5μ (22 by 4).
2-septate, rare.
1-septate, 9 per cent, 20 by 3μ (1).
0-septate, 67 per cent, 7.5 by 2.5μ (1).
- On onion-agar tubes, culture 27 days old, conidia from sporodochium:
5-septate, 2 per cent, 49.8 by 4.15μ average (10), 44.8 to 53.1 by 3.32 to 4.98μ .
4-septate, 17 per cent, 44.3 by 4.1μ average (10), 38.2 to 47.3 by 3.32 to 4.98μ .
3-septate, 68 per cent, 41.1 by 3.8μ average (10), 33.2 to 46.4 by 3.32 to 4.15μ .
Small 3-septate, 25.7 by 2.7μ average (10), 23.2 to 28.2 by 2.5 to 2.9μ .
2-septate, rare, 16.6 by 3.32μ (1).
1-septate, 2 per cent, 12.8 by 2.7μ average (10), 8.3 to 19.9 by 1.66 to 3.32μ .
0-septate, 11 per cent, 6.5 by 2.8μ average (10), 5 to 10 by 2.5 to 3.32μ .

On cornmeal agar, culture 9 days old, conidia from very thin pionnotes:

Macroconidia 99 per cent.

3-septate in majority.

4-septate and 5-septate common.

0 and 1 septate 1 per cent.

Average of all media:

6-septate, 52.4 by 3.3μ average (5), 46.8 to 55 by 2.5 to 3.7μ .

5-septate, 44.4 by 3.9μ average (39), 38 to 53 by 2.7 to 5μ .

4-septate, 40.9 by 3.7μ average (55), 32 to 51 by 3 to 5μ .

3-septate, 35.7 by 3.8μ average (110), 25 to 46 by 3 to 4.5μ .

Small 3-septate, 25.7 by 2.7μ average (10), 22.5 to 28 by 2 to 3μ .

2-septate, 17.4 by 2.8μ average (6), 16.5 to 17.6 by 2 to 3μ .

1-septate, 12.7 by 2.75μ average (26), 11.5 to 17 (20) by 2 to 3μ .

0-septate, 7.5 by 2.7μ average (35), 6 to 9 (13) by 2 to 3.25μ .

Some of the other organisms of the original pionnotes group proved identical. These were 1456-c, 1458-a, 1443-b, 1475-d, and 1454-b, all from onions from Walla Walla, Wash. Culture 1456-c was used in identification and was found to be identical with 1490, *F. zonatum* forma 1, in every respect except that in place of the salmon to salmon buff it constantly developed a lavender to red color on certain media. It is described as *F. zonatum* (Sherb.) Wr. forma 2 (nova forma).

The morphological and cultural characters of the organism were determined by cultures on various media and are as follows:

F. ZONATUM (SHERB.) WR. FORMA 2, NOVA FORMA

Fine, fluffy white to purplish lilac mycelium, typically scant on most media; substratum on oatmeal agar plates dull Indian purple; no sclerotia but small ($1/2$ mm.) warm sepia to warm blackish brown plectenchymatic cushions present on steamed rice, these sometimes giving rise to spore masses; color on rice Acajou red to Indian lake with light vinaceous lilac to carmine mycelium at top; slight lilac odor on rice; chlamydospores (fig. 8, C, b) abundant in spores and mycelium, terminal and intercalary, unicellular to two celled, solitary, in chains or in groups, smooth to echinate; microconidia (fig. 8, E) scattered in aerial mycelium or present in sporodochia or pionnotes, 0-septate mostly 7.9 by 2.8μ average (5 to 11.6 by 1.7 to 4.56μ), some 1-septate 14 by 3μ average (8.3 to 18.3 by 1.7 to 4.56μ); macrospores (fig. 8, A-E) borne on irregularly branched sporophores (fig. 8, D) in sporodochia and pionnotes, vinaceous cinnamon to buff pink or light ochraceous salmon, sickle-shaped, pedicellate, slightly constricted at apex, 3- to 6-septate, predominately 3-septate, 38.5 by 3.7μ average (24 to 48 by 2.4 to 4.56μ), on potato-tuber plugs an occasional small 3-septate spore (fig. 8, A, a) 19 by 2.8μ average (16.6 to 25 by 2.5 to 3.32μ); 2-septate rare, 18.1 by 3.2μ average (15 to 21.6 by 2.9 to 4.15μ); 4-septate 39.9 by 3.8μ average (31.5 to 53 by 3.2 to 4.56μ); 5-septate, 42.1 by 4.1μ average (36.5 to 50 by 3.2 to 5μ). Zonation sometimes marked on agar plates.

Differs from *Fusarium zonatum* forma 1 by the constant red color on rice and the lilac mycelium and vinaceous cast present in spore masses.

LATIN DESCRIPTION

Aerio mycelio subtile, ex albo vel "purplish lilac," plerumque exiguo; sporodochiis et pionnote; substrato in Avenae coctae agare "dull Indian purple," in acida agare *Solani tuberosi* "light perilla purple"; sclerotiiis nullis, sed parvis $\frac{1}{2}$ millimeter "warm sepia" vel "warm blackish brown" plectenchymicis pulvinis in oryza cocta, interdum sporodochia ferentibus; colore in oryza cocta "Acajou red" vel "Indian lake" cum "light vinaceous lilac" vel "carmine" mycelio in apice; parvo odore Syringae in oryza cocta; chlamydosporiis (fig. 8, C b) largis inconidiis et mycelio, terminalibus aut intercalaribus, continuis, 1-septatis, singulis, catenulatis, aut acervalibus, levibus vel rugulosis; microconidiis (fig. 8, E) aerio mycelio instratis, aut in sporodochiis vel in pionnote dispositis, ellipsoideo-ovoides vel vix dorsiventralibus; plerumque continuis, 7.9 by 2.8μ average (5 to 11.6 by 1.7 to 4.56μ); 1-septate paucis 14 by 3μ average (8.3 to 18.3 by 1.7 to 4.56μ); macroconidiis (fig. 8 A-E) ad conidiophoros (fig. 8, D) irregulariter ramosos dispositis, liberis, in sporodochiis et in pionnote, "vinaceous

cinnamon," "buff pink," aut "light ochraceous salmon," falcatis, pedicellatis, apice leviter constricto; 3 to 6 septate; plerumque 3-septate 38.5 by 3.7μ average (24 to 48 by 2.4 to 4.56μ); in tuberibus Solani tuberosi aliquando minoribus 3-septate conidiis (fig. 8, A, a) 19 by 2.8μ average (16.6 to 25 by 2.5 to 3.32μ);

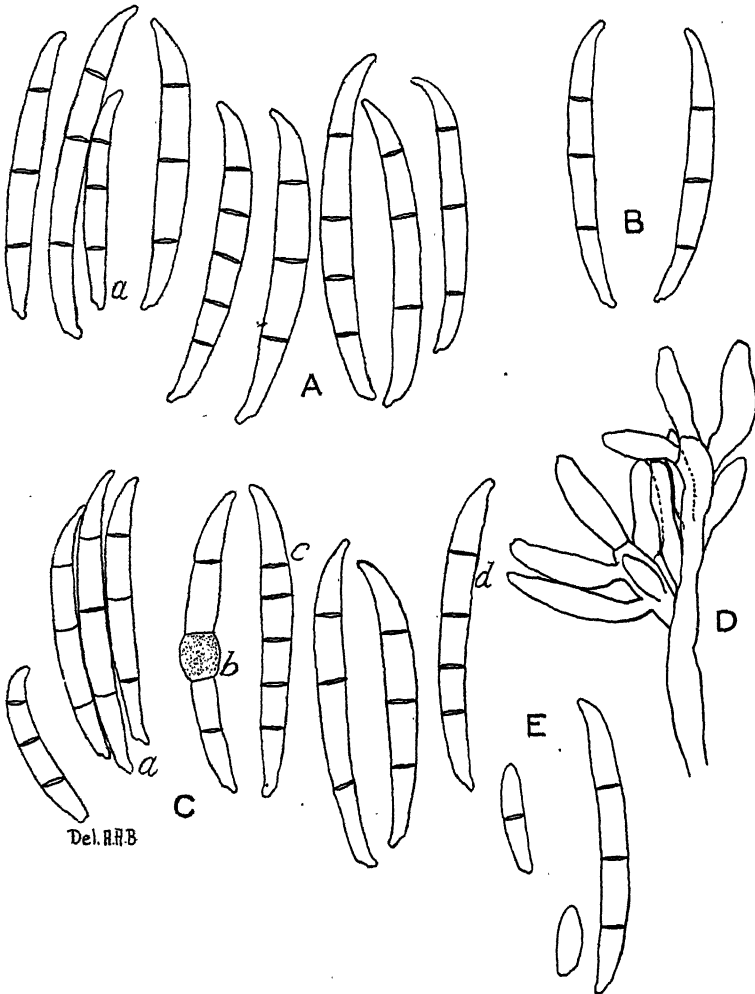


FIG. 8.—Spores of 1456-c, *Fusarium zonatum* (Sherb.) Wr. forma 2, nova forma

- A.—Macrospores from dry sporodochium on top of 37-day-old steamed rice culture; a, small 3-septate spore
 B.—Macrospores from pionnotes on 35-day-old potato-tuber plug culture
 C.—Macrospores from pionnotes on 35-day-old potato-agar tube culture: a, 3-septate spores; b, Chlamydospore in macroconidium; c, 5-septate spore; d, 4-septate spore
 D.—Sporophore from pionnotes on 13-day-old *Melilotus* stem culture
 E.—Microspores and macrospore from pionnotes on 13-day-old *Melilotus* stem culture
 Camera lucida drawings. All $\times 1,000$

raro 2-septate, 18.1 by 3.2μ average (15 to 21.6 by 2.9 to 4.15μ); 4-septate 39.9 by 3.8μ average (31.5 to 53 by 3.2 to 4.56μ); 5 septate, 42.1 by 4.1μ average (36.5 to 50 by 3.2 to 5μ); zonae interduum apertae in agar.

A. *F. zonata* forma differt constante rubro colore in *Oryza coctia*, ferme purpureo substrato, "lilac" mycelio, et "vinaceous" colore in conidiorum molibus.

Measurements of spores on different media are as follows:

- On potato-dextrose-agar tube, culture 35 days old, conidia from pionnotes:
 5-septate, 8 per cent, 42.5 by 4.3μ average (5), 41 to 43 by 4 to 4.5μ .
 4-septate, 37 per cent, 41 by 4μ average (10), 38 to 43 by 3.75 to 4.5μ (23 by 3, 45 by 4μ).
 3-septate, 53 per cent, 40.1 by 4μ average (10), 36 to 43 by 3.75 to 4μ (48 by 4).
 1-septate, 1 per cent.
 0-septate, 1 per cent.
- On potato-dextrose-agar tube, culture 90 days old, conidia from pionnotes:
 6-septate, 2.7 per cent.
 5-septate, 18.7 per cent.
 4-septate, 27.0 per cent.
 3-septate, 47.9 per cent.
 0-septate, 3.7 per cent.
- On oatmeal-agar tube, culture 26 days old, conidia from pionnotes:
 4-septate, 4.7 per cent, 46.3 by 4.15μ average (10), 41.5 to 53 by 3.73 to 4.56μ .
 3-septate, 89.9 per cent, 43 by 3.7μ average (10), 40 to 45.6 by 3.32 to 4.15μ .
 2-septate, rare, 21.6 by 4.15μ (1).
 1-septate, 1.8 per cent, 20 by 3.32μ (1).
 0-septate, 3.6 per cent, 8 by 2.7μ average (5), 5 to 11.6 by 2.5 to 3.32μ .
- On oatmeal-agar plate, culture 36 days old, conidia from pionnotes:
 5-septate, 5.4 per cent, 40 by 3.4μ average (2), 40 by 3.2 to 3.6μ .
 4-septate, 18.4 per cent, 37.8 by 3.2μ average (3), 36.8 to 38.4 by 3.2μ .
 3-septate, 61.6 per cent, 33.6 by 2.9μ average (10), 32 to 36.8 by 2.4 to 3.6μ .
 0-septate, 14.6 per cent.
- On potato-tuber plug, culture 18 days old, conidia from sporodochium:
 6-septate, 0.4 per cent.
 5-septate, 0.4 per cent, 39 by 3.7μ average (2), 37 to 40.7 by 3.7μ .
 4-septate, 0.4 per cent, 37.3 by 3.73μ average (9), 33.3 to 42.5 by 3.7 to 4.16μ .
 3-septate, 93 per cent, 34 by 3.7μ average (15), 29.6 to 40.7 by 3.7 to 4.16μ .
 2-septate, 0.4 per cent.
 1-septate, 1.4 per cent.
 0-septate, 4.0 per cent.
- On potato-tuber plug, culture 35 days old, conidia from pionnotes:
 4-septate, 6 per cent, 42 by 4μ (1).
 3-septate, 94 per cent, 37.3 by 3.5μ average (3), 36 to 39 by 3.5μ .
- On potato-tuber plug, culture 37 days old, conidia from sporodochium:
 6-septate, rare, 38.8 by 4.15μ (1).
 5-septate, 15 per cent, 40 by 4μ average (10), 36.5 to 45 by 3.32 to 4.15μ .
 4-septate, 29.4 per cent, 38.6 by 4μ average (10), 31.5 to 41.5 by 3.32 to 4.15μ .
 3-septate, 24.5 per cent, 36.6 by 4μ average (10), 33.2 to 41.5 by 3.32 to 4.15μ .
 Small 3-septate, 6.9 per cent, 19 by 2.8μ average (10), 16.6 to 25 by 2.5 to 3.32μ .
 2-septate, 2.6 per cent, 17 by 2.9μ average (3), 15 to 20 by 2.9μ .
 1-septate, 5.2 per cent, 13.4 by 2.7μ average (10), 8.3 to 16.6 by 1.7 to 3.32μ .
 0-septate, 16.4 per cent, 7.5 by 2.8μ average (10), 5.8 to 10 by 1.7 to 3.32μ .
- On Melilotus stems, culture 26 days old, conidia from pionnotes:
 From thick pionnotes at moist bottom of tube: 3-septate, 38 per cent;
 0-septate, 62 per cent.
 From thin pionnotes on dry upper portion: 3-septate, 64.8 per cent; 2-septate, 2.3 per cent; 1-septate, 5.3 per cent; 0-septate, 27.6 per cent.
 3-septate, 39.3 by 3.2μ average (10), 24 to 41.6 by 2.8 to 3.6μ .
 1-septate, 16 by 2μ (1).
 Chlamydospore 6.4 by 6.4μ (1).
- On onion-agar tube, culture 20 days old, conidia from pionnotes:
 5-septate, 6.5 per cent, 45 by 4.4μ average (10), 41.5 to 50 by 3.7 to 5μ .
 4-septate, 20.4 per cent, 45.2 by 4.23μ average (10), 41.5 to 50 by 3.32 to 4.56μ .
 3-septate, 66.1 per cent, 45.3 by 4.15μ average (10), 41.5 to 48 by 3.32 to 4.56μ .
 1-septate, 2 per cent, 13.7 by 3.32μ average (10), 10 to 18.3 by 2.9 to 4.56μ .
 0-septate, 5 per cent, 8.3 by 3μ average (10), 5 to 11 by 2 to 4.56μ .
- On steamed rice, culture 37 days old, conidia from dry sporodochium:
 4-septate, 3 per cent, 47.5 by 4.4μ average (2), 47 to 48 by 4.25 to 4.5μ .
 3-septate, 89 per cent, 39 by 3.73μ average (15), 35 to 43 by 3.5 to 4.5μ (30 by 3.47 by 3.5μ).
 1-septate, 1 per cent.
 0-septate, 7 per cent.

On 20 per cent dextrose-agar plate, culture 50 days old, conidia from pionnotes:

- 4-septate, 6.7 per cent.
- 3-septate, 64.2 per cent.
- 1-septate, 0.6 per cent.
- 0-septate, 28.5 per cent.

Average of all media:

- 6-septate, 39.8 by 4.15μ average (1).
- 5-septate, 42.1 by 4.1μ average (29), 36.5 to 50 by 3.2 to 5μ .
- 4-septate, 39.9 by 3.8μ average (55), 31.5 to 53 by 3.2 to 4.56μ .
- 3-septate, 38.5 by 3.7μ average (93), 24 to 48 by 2.4 to 4.56μ .
- Small 3-septate, 19 by 2.8μ average (10), 16.6 to 25 by 2.5 to 3.32μ .
- 2-septate, 18.1 by 3.2μ average (4), 15 to 21.6 by 2.9 to 4.15μ .
- 1-septate, 14 by 3μ average (22), 8.3 to 18.3 by 1.7 to 4.56μ .
- 0-septate, 7.9 by 2.8μ average (25), 5 to 11.6 by 1.7 to 4.56μ .

Of the 10 cultures studied critically, 1324 (Delta, Colo.), 1626-b (Walla Walla, Wash.), 1455-d (Walla Walla, Wash.), 1495 (Delta, Colo.), and 1407 (Stockton, Calif.) were found nonpathogenic. No. 1324 is *F. vasinfectum* Atk., 1626-b is *F. oxysporum* (Schlecht.) Wr., 1455-d is *F. bulbigenum* Cke. et Mass., 1495 is *F. martii*, Appel et Wr. var. *minus* Sherb., and 1407 may be *F. malli* Tauben. No positive diagnosis has yet been made of 1407 which belongs to group Martiella because the fungus has consistently refused to produce macrospores. Cultures 840-b and 1624-b' are *F. monili-forme* Shield. and are pathogenic sometimes. (See Table 4.)

During the course of the identification of the organisms nine authentic cultures were obtained through the courtesy of H. W. Wollenweber. These and 15 additional authentic cultures were used in inoculation work with the results shown in Table 6.

Fusarium cepae rots bulbs, whereas inoculations with cultures of *F. aurantiacum* and *F. sclerotioides* proved negative; this table and Table 4 indicate that the Fusaria which cause bulb rot are specialized pathogenes. Other members of the form genus *Fusarium* which occur commonly in the soil, or which occur on rotted bulbs in association with bacteria or the true pathogenes, fail to produce rot under experimental conditions.

TABLE 6.—Inoculation with authentic *Fusarium* cultures

Culture No.	Name of organism	Where obtained ^a	Number of inoculations	Number of bulbs decayed
1592.....	<i>F. batatatis</i>	S	3	0
1525.....	<i>F. coeruleum</i>	S	3	0
1594.....	<i>F. conglutinans</i>	J	3	0
1523.....	<i>F. discolor</i>	S	3	0
1506.....	<i>F. eumartii</i>	S	3	0
1611.....	<i>F. hyperoxysporum</i>	H	3	0
1593.....	<i>F. hyperoxysporum</i>	H	3	0
1533.....	<i>F. hyperoxysporum</i>	H	3	0
1518.....	<i>F. lycopersici</i>	S	3	0
1505.....	<i>F. oxysporum</i>	S	3	0
1507.....	<i>F. radiclecola</i>	S	5	2
1534.....	<i>F. solani</i>	S	3	0
1520.....	<i>F. sulphureum</i>	S	3	0
1522.....	<i>F. vasinfectum</i>	S	3	0
1625.....	<i>F. redolens</i>	W	2	0
1629.....	<i>F. orthoceros</i>	W	2	0
1630.....	<i>F. asclerotium</i>	W	2	0
1631.....	<i>F. sclerotioides</i>	W	2	0
1632.....	<i>F. zonatum</i>	W	4	0
1633.....	<i>F. oxysporum</i>	W	2	0
1634.....	<i>F. bulbigenum</i>	W	2	0
1635.....	<i>F. aurantiacum</i>	W	2	0
1636.....	<i>F. orthoceros</i> var. <i>longius</i>	W	2	0
1660.....	<i>F. malli</i>	N	5	2

^a H, L. L. Harter; J, Helen Johann; N, A. G. Newhall at Cornell; S, Michael Shapovalov; W, H. W. Wollenweber.

^b Slight decay, but not typical rot.

^c Rot + bacteria.

SUMMARY

A bulb rot of onions caused by *Fusarium* species occurs widely in the field and after harvest in transit and in storage.

Red, yellow, and white varieties are equally susceptible. Decay was readily produced when bulbs were inoculated in wounds.

The *Fusaria* which cause onion bulb rot apparently are specialized pathogenes attacking only *Allium* species.

Several species of *Fusarium* were found to cause identical symptoms. Most of these species belong to section *Elegans*. In this paper are described *F. zonatum* (Sherb.) Wr. forma 2, nova forma and *F. cepae* (Hanzawa) emend. (non *F. cepae* (Hanzawa) emend. Walker and Tims). Another species of this section which is always pathogenic is *F. zonatum* (Sherb.) Wr. forma 1.

Fusarium bulbigenum Cke. et Mass. of section *Elegans* is not consistently pathogenic. *F. oxysporum* (Schlecht.) Wr. and *F. vasinfectum* Atk. are not pathogenic.

Fusarium moniliforme Sheld. of section *Liseola* is pathogenic at times.

Not all *Fusaria* isolated from decaying bulbs are pathogenic in pure culture. Some of them cause symptoms similar to bulb rot when in association with bacteria. Most of these belong to section *Elegans* and to section *Martiella*. Some belong to sections *Discolor* and *Liseola*.

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THE ALTERNATE HOSTS OF CROWN RUST, PUCCINIA CORONATA CORDA¹

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INTRODUCTION

As has been shown previously (9, 4),³ many species of the genus *Rhamnus* are susceptible to crown rust (*Puccinia coronata* Corda) and play an important rôle in the dissemination of this disease in America. The universal presence of the uredinal stage of this rust in certain regions where no *Rhamnus* is found or only resistant species occur led the writer to a further study of the range of the aecial hosts of this fungus.

European investigators have been interested in the alternate hosts of crown rust since 1866 when De Bary (3) discovered that *Rhamnus cathartica* L. harbored the aecial stage. An earlier résumé (9) of the literature on the alternate hosts of *Puccinia coronata* and their ability to spread rust in the open (4) makes unnecessary a review in this paper.

The reaction of 16 species and 1 variety of *Rhamnus* to crown rust, the testing of 4 genera other than *Rhamnus*, and the determination of the rôle of *R. cathartica* L. and *R. lanceolata* Pursh. in initiating local and general epidemics of *Puccinia coronata* in the upper Mississippi Valley are considered in this paper.

MATERIALS AND METHODS

During the past six years studies have been made in the greenhouse and laboratories of the Iowa Agricultural Experiment Station at Ames, Iowa, to determine the reaction of 16 species and 1 variety of *Rhamnus*, and 5 species of 4 other genera, to crown rust. Succulent young leaves were inoculated with germinating teliospores from *Avena sativa* L., *Calamagrostis canadensis* (Michx.) Beauv., *C. purpurascens* R. Br., *Festuca elatior* L., and *Notholcus lanatus* (L.) Nash. (*Holcus lanatus* L.). The species of *Rhamnus* and other genera were exposed to infection by placing them in a moist chamber with teliospore-laden straw. The plants, straw, and sides of the chamber were then wet by a fine spray. In cases where scant spore material was available, the spores were germinated on the straw in Petri dishes as described by Melhus and Durrell (8). Later, the sporidia were washed off and sprayed onto the plants with an atomizer. If the chamber showed signs of becoming dry before the close of the incubation period, it was opened and sprayed again. This

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³ Reference is made by number (italic) to "Literature cited." p. 969.

method successfully induced infection. At the end of two days the plants were removed to the greenhouse bench, where they were observed for the appearance of rust.

SOURCE AND CARE OF ALTERNATE HOSTS

Five additional native and two European species of *Rhamnus* have become available for alternate-host studies since 1922. Species of *Ceanothus*, *Berchemia*, and *Zizyphus*, of the family Rhamnaceae, together with two species of *Lepargyrea*, of the family Eleagnaceae, have been studied to determine their reaction to germinating teliospores of *Puccinia coronata*.

Rhamnus dahurica Pallas is a cultivated shrub indigenous to the territory between India and northern Asia. Its bark is used in the manufacture of Chinese green indigo. Because of its extreme hardness, this shrub is becoming common for landscaping in certain sections of America where the winters are severe. The material used here was grown from seed collected on the campus at Ames, Iowa.

Rhamnus ilicifolia Kellogg is native to southern and middle California, and is especially abundant on Angel Island, in San Francisco Bay, where it often reaches a height of 10 to 12 feet. It is very similar to *R. crocea*, but differs from the latter in its arboreal habit, gray bark, and larger leaves and fruit. This material was obtained in California.

Rhamnus nevadensis Nelson is indigenous to Nevada. It is very similar to *R. californica* Esch., but has noncoriaceous leaves and short calyx lobes. It seems much hardier under Iowa conditions. The one plant available in this investigation was found in Nevada.

Rhamnus pachyphylla Heller, native to California, has a limited distribution. Its leaf is thick and leathery with an entire margin. *R. pinetorum* Heller also has a rather limited distribution in California.

Rhamnus rubra Greene is listed by Trelease (14) as a variety of *R. californica*. Greene (6, p. 160), however, thinks it a good species, since its seeds are only half as large as those of *R. californica* and also are decidedly narrowed at the base. The plants used in these experiments had bark of a deeper red than those of *R. californica*. This species is common in California, especially in Butte County between Upper Soda Springs and Shasta Retreat. The writer is indebted to A. A. Heller for the collection of *R. pachyphylla*, *R. pinetorum*, and *R. californica*.

Rhamnus tinctoria Waldst. and Kit. is native to Europe and Asia Minor, where it is used in the manufacture of dye.

Berchemia scandens (Hill) Trel. is a climbing plant growing in damp soils from Virginia to Missouri and southward. The stems are tough and lithe, hence the popular name, supple-jack. This material was collected in southern Alabama.

Ceanothus americanus L., commonly called New Jersey tea, grows in dry woodlands, along gravelly shores and sandy knolls, from central Maine to western Ontario and southward through Iowa. The material employed in these studies was collected 3 miles north of Ames, Iowa, on virgin prairie land.

Lepargyrea argentea Greene is widely distributed in the northeastern part of the United States and Canada, extending southward

to northern New Mexico in the Rocky Mountain region. *L. canadensis* Greene is even more extensive in distribution than *L. argentea*. It extends across southern Canada and up to Alaska. In the Rocky Mountain region, however, temperature seems to limit its southern distribution, as this shrub could be found only at an elevation in excess of 7,500 feet near Bergen Park, in Colorado. The materials used in these investigations were collected at Pagosa Spring and Bergen Park, Colo.

Zizyphus lycioides Gray, a spiny shrub with zigzag branches, is native to the southern part of the United States. The material used was collected in southern Alabama.

Considerable difficulty was experienced at first in overwintering these plants. As an extremely succulent growth was needed for inoculation during six weeks in the spring, the question arose as to the best method of maintaining these plants throughout the remainder of the year. Four methods, all unsatisfactory at Ames, were as follows: (1) Maintenance under greenhouse conditions throughout the year; (2) heeling-in during the winter out of doors and transferring to the greenhouse in the spring; (3) packing in moss and keeping in cold storage during the winter; (4) cuttings.

Two methods of obtaining succulent spring growth were successfully used at Ames. In the first, the plants were set in 12-inch pots placed outdoors in June, and set 3 inches below the ground line. They were allowed to harden off in the fall until defoliated, after which they were covered with 6 inches of straw and allowed to remain outside until the first of March. They were then kept in a cold room (45° to 55° F.) for three weeks and subsequently placed on the greenhouse bench (temperature 65° to 70°). It seems important that these plants be started slowly when brought into the greenhouse, as in previous years over 50 per cent of them died when taken directly from the outside into a heated room.

A second satisfactory method was to sow *Rhamnus* seed in the greenhouse in November. Seeds of *R. dahurica*, *R. lanceolata*, *R. cathartica*, *R. californica*, *R. purshiana*, and *R. alnifolia* germinated strongly if gathered in the fall and sown before the fruit became dry.

After the plants had become established on the greenhouse bench in the spring, two methods of obtaining succulent growth were used: (1) The plants were watered daily with liquid manure and the temperature raised to 75° to 80° F.; (2) the seedlings, which had been maintained under greenhouse conditions, were severely pruned and then forced by means of fertilizer and heat.

SOURCE AND CARE OF TELIOSPORES

During 1923, teliospores from *Avena sativa* L. were collected from four places in Iowa and Illinois. These had a low percentage of germination. Those collected at Ames and Masonville, Iowa, during 1924, germinated with greater vigor.

Teliospores were collected from *Calamagrostis canadensis* in Iowa, Wisconsin, and Canada. Those from Iowa germinated between 90 and 100 per cent, but the other collections had only a weak germination. Teliospores were collected at Eldora Springs, Colo., from *C. purpurascens*.

A collection of teliospores from *Festuca elatior* was made at Ames, Iowa. Teliospores were collected from greenhouse cultures of *Notholcus lanatus* but germinated poorly.

The germination of teliospores in the spring was influenced by the method of overwintering the host plants. Those from *Avena sativa*, *Festuca elatior*, and *Notholcus lanatus* germinated most satisfactorily when the host plant was left in the field and the spore material collected in the spring, while those on *Calamagrostis canadensis* germinated equally well whether collected in the fall and wrapped in cheesecloth or obtained from the host plant in the spring.

TYPES OF INFECTION OF ALTERNATE HOSTS

Successful attempts to infect *Rhamnus* species developed the following four types of aecial infection: (1) Necrotic areas, no sporulation; (2) normal pycnia, no aecia; (3) normal pycnia, and few aecia; (4) normal pycnia and aecia.

In type 1 the germ tube of the sporidium caused etiolated, light yellow areas, usually surrounding a dead center with no form of sporulation. In type 2, there was normal pycnial development but little or no evidence of aecia. Type 3 showed normal pycnia but few aecia. Type 4 was characterized by profuse development of normal pycnia and aecia.

Growth factors, such as relative humidity and temperature, played an important part in determining whether an infection of type 3 or 4 would develop. A high humidity and a temperature of about 20° C. caused profuse development of aecial cups on susceptible *Rhamnus* species. Inherent rather than environmental causes were probably more responsible in determining whether aecial infection was of type 2 or 3.

THE ALTERNATE HOSTS OF CROWN RUST

It has been shown earlier (9) that the uredinial hosts of *Puccinia coronata* are not restricted to one genus. Whether the alternate host of crown rust was limited to the genus *Rhamnus* has not previously been known. To determine this, the following genera were exposed to infection from teliospores of crown rust: *Berchemia*, *Ceanothus*, *Lepargyrea*, *Rhamnus*, and *Zizyphus*.

THE GENUS RHAMNUS

The results obtained by exposing 16 species and 1 variety of *Rhamnus* to crown-rust infection 364 times are presented in Table 1. Teliospores from *Avena sativa*, *Calamagrostis canadensis*, and *Festuca elatior* were used, as these gramineous hosts are generally present throughout the main oat-growing sections of the upper Mississippi Valley.

TABLE 1.—*Response of Rhamnus species to inoculation with teliospores of Puccinia coronata from various grasses, in the years 1919 to 1924, inclusive*

Rhamnus species	Teliospores from—	Times inoculated	Types of infection				
			None	Flecks	Pyenia	Few aecia	Normal
<i>Alnifolia</i>	<i>Avena sativa</i>	12	7	1	—	4	—
Do.....	<i>Calamagrostis canadensis</i>	15	2	1	—	—	12
Do.....	<i>Festuca elatior</i>	2	1	—	—	—	1
<i>Californica</i>	<i>Avena sativa</i>	13	4	—	—	9	—
Do.....	<i>Calamagrostis canadensis</i>	18	1	1	7	8	1
Do.....	<i>Festuca elatior</i>	4	2	—	1	—	1
<i>Californica</i> var. <i>tomentella</i>	<i>Avena sativa</i>	7	2	—	—	2	3
Do.....	<i>Calamagrostis canadensis</i>	13	3	4	6	—	—
Do.....	<i>Festuca elatior</i>	4	2	—	2	—	—
<i>Caroliniana</i>	<i>Avena sativa</i>	13	7	—	3	1	2
Do.....	<i>Calamagrostis canadensis</i>	11	5	2	4	—	—
Do.....	<i>Festuca elatior</i>	2	—	—	—	2	—
<i>Cathartica</i>	<i>Avena sativa</i>	26	2	—	—	—	24
Do.....	<i>Calamagrostis canadensis</i>	24	14	5	3	2	—
Do.....	<i>Festuca elatior</i>	5	—	—	—	—	5
<i>Crocea</i>	<i>Avena sativa</i>	1	—	—	—	1	—
Do.....	<i>Calamagrostis canadensis</i>	6	—	—	—	—	6
Do.....	<i>Festuca elatior</i>	1	—	—	—	1	—
<i>Dahurica</i>	<i>Avena sativa</i>	13	5	1	3	4	—
Do.....	<i>Calamagrostis canadensis</i>	6	5	—	1	—	—
Do.....	<i>Festuca elatior</i>	2	2	—	—	—	—
<i>Frangula</i>	<i>Avena sativa</i>	17	8	7	2	—	—
Do.....	<i>Calamagrostis canadensis</i>	23	23	3	2	—	—
Do.....	<i>Festuca elatior</i>	3	3	—	—	—	—
<i>Illicifolia</i>	<i>Avena sativa</i>	4	2	—	—	—	2
Do.....	<i>Calamagrostis canadensis</i>	3	3	—	—	—	—
Do.....	<i>Festuca elatior</i>	1	—	1	—	—	—
<i>Lanceolata</i>	<i>Avena sativa</i>	11	2	—	—	—	9
Do.....	<i>Calamagrostis canadensis</i>	24	5	1	—	4	14
Do.....	<i>Festuca elatior</i>	3	—	—	—	—	3
<i>Nevadensis</i>	<i>Avena sativa</i>	1	1	—	—	—	—
Do.....	<i>Calamagrostis canadensis</i>	0	—	—	—	—	—
Do.....	<i>Festuca elatior</i>	0	—	—	—	—	—
<i>Pachyphylla</i>	<i>Avena sativa</i>	5	1	—	3	—	1
Do.....	<i>Calamagrostis canadensis</i>	4	—	—	4	—	—
Do.....	<i>Festuca elatior</i>	1	—	1	—	—	—
<i>Pinetorum</i>	<i>Avena sativa</i>	3	1	—	—	—	2
Do.....	<i>Calamagrostis canadensis</i>	2	—	—	—	2	—
Do.....	<i>Festuca elatior</i>	1	1	—	—	—	—
<i>Purshiana</i>	<i>Avena sativa</i>	8	3	3	2	—	—
Do.....	<i>Calamagrostis canadensis</i>	10	7	2	1	—	—
Do.....	<i>Festuca elatior</i>	3	3	—	—	—	—
<i>Rubra</i>	<i>Avena sativa</i>	5	—	—	—	4	1
Do.....	<i>Calamagrostis canadensis</i>	6	—	—	—	6	—
Do.....	<i>Festuca elatior</i>	2	1	—	—	1	—
<i>Smithii</i>	<i>Avena sativa</i>	7	1	—	—	2	4
Do.....	<i>Calamagrostis canadensis</i>	10	1	—	—	—	9
Do.....	<i>Festuca elatior</i>	1	—	—	—	1	—
<i>Tinctoria</i>	<i>Avena sativa</i>	1	—	—	—	—	—
Do.....	<i>Calamagrostis canadensis</i>	1	—	—	1	—	—
Do.....	<i>Festuca elatior</i>	1	1	—	—	—	—

When inoculated with teliospores from *Avena sativa*, it was found that *Rhamnus californica* var. *tomentella*, *R. caroliniana*, *R. cathartica*, *R. illicifolia*, *R. lanceolata*, *R. pachyphylla*, *R. pinetorum*, *R. rubra*, and *R. smithii* were susceptible, producing type-4 infection, and that *R. alnifolia*, *R. californica*, *R. crocea*, *R. dahurica*, and *R. tinctoria* produced a type-3 infection (figs. 1 and 2). *R. frangula*, *R. nevadensis*, and *R. purshiana* showed a marked resistance to this physiologic form of rust. *R. dahurica* probably possesses inherent resistance (fig. 2), as the aecial sori were small, and contained only a few cluster cups. More inoculations are necessary to determine the true response of *R. nevadensis*, as only one plant was available in the experiments here recorded. When inoculated with teliospores from *Calamagrostis canadensis*, *R. alnifolia*, *R. californica*, *R. crocea*, *R. lanceolata*, and *R.*

smithii were infected normally. In addition, *R. cathartica*, *R. pine-torum*, and *R. rubra* may function as alternate hosts for this form, as they produced a type-3 infection. The other eight species of *Rhamnus* showed a marked resistance.

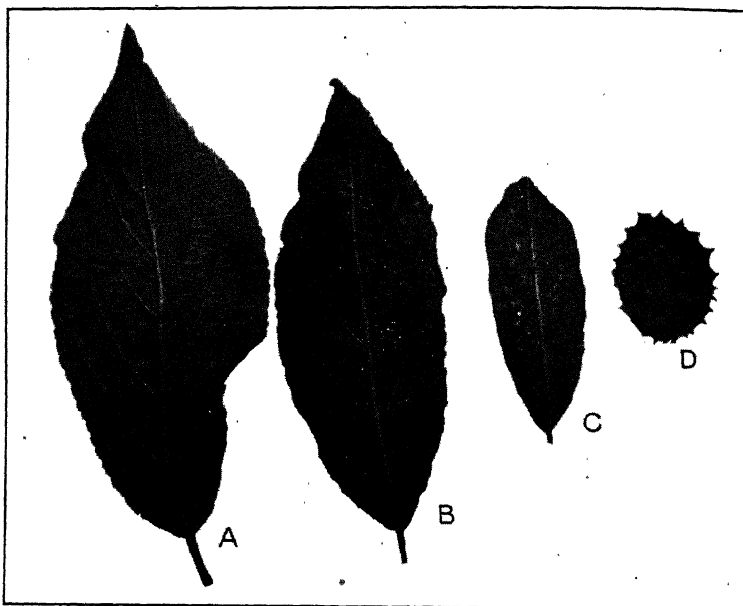


FIG. 1.—Infection from *Arena sativa* on *Rhamnus* species. A, *Rhamnus alnifolia*; B, *R. caroliniana*; C, *R. rubra*; D, *R. ilicifolia*

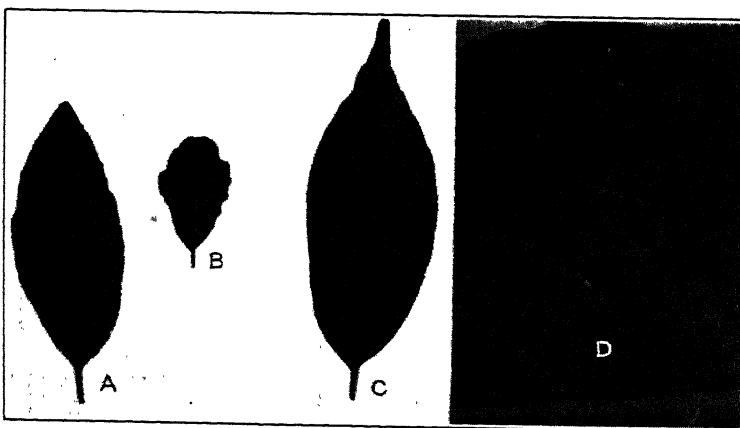


FIG. 2.—Infection from *Arena sativa* to *Rhamnaceae*. A, *Rhamnus smithii*; B, *R. pinetorum*; C, *R. lanceolata*; D, *R. dahurica*. $\times 3$

Teliospores from *Festuca elatior* caused normal infection on *Rhamnus alnifolia*, *R. californica*, *R. cathartica*, and *R. lanceolata*. On *R. caroliniana*, *R. crocea*, *R. rubra*, and *R. smithii* a few aecia were produced. Twelve inoculation trials on the seven remaining *Rhamnus* species

showed that *R. ilicifolia* produced pycnia and *R. pachyphylla* became flecked. Owing to the limited teliospore material, further investigation is necessary to determine the reponse of *Rhamnus* species to crown rust from *F. elatior*.

A limited inoculum of teliospores from *Notholcus lanatus* was transferred to *Rhamnus alnifolia*, *R. californica*, *R. californica* var. *tomentella*, *R. cathartica*, *R. caroliniana*, *R. crocea*, *R. frangula*, *R. lanceolata*, *R. purshiana*, and *R. smithii*. *R. lanceolata* produced pycnia. Further tests are necessary.

It should be noted that the reaction of the *Rhamnus* species to crown rust from *Festuca elatior* paralleled closely that resulting from the strain from *Avena sativa*. A greater divergence between the reaction of the *A. sativa* and *Calamagrostis canadensis* strains is apparent. The reaction of *R. caroliniana* to the strain from *A. sativa* is particularly noticeable, as this species was infected normally but twice in 13 attempts. The normal infection occurred during 1924, the most favorable year for obtaining infection in the six years of experimentation. Although *R. frangula* was inoculated 48 times and *R. purshiana* 21 times, both maintained a marked resistance to crown rust from the three gramineous hosts.

Many of the *Rhamnus* species contained some individuals which showed no infection and others which showed normal infection (Table 1). This variation probably was not always due to an inherent difference in the individuals, as some of the same plants developed no infection at one inoculation and normal infection during a subsequent attempt. The species were divided into susceptible and resistant groups. All that functioned as alternate hosts by producing aecia were classified as susceptible.

TABLE 2.—Division of *Rhamnus* species into susceptible and resistant groups according to types of infection

Inoculated with teliospores from—	Susceptible species							Resistant species						
	Number of species	Number of trials	Types of infection					Number of species	Number of trials	Types of infection				
			None	Flecks	Pycnia	Few aecia	Normal			None	Flecks	Pycnia	Few aecia	Normal
<i>Avena sativa</i>	14	121	34	2	9	28	48	3	26	12	10	4	0	0
<i>Calamagrostis canadensis</i>	*9	108	26	12	10	28	32	*7	63	43	7	13	0	0
<i>Festuca elatior</i>	*9	24	6	0	1	7	10	*7	12	10	1	1	0	0
Total.....	---	253	66	14	20	63	90	---	101	65	18	18	0	0

* *Rhamnus nevadensis* was exposed to infection only with teliospores from *Avena sativa*.

The production of a single aecium was considered indicative of the inherent susceptibility of a species, and when further trials produced only negative results it was held to be due probably to the environmental conditions of the plant during inoculation or to possible nonviability of the teliospores. As shown in Table 2, the mode of each dispersion is at the normal class when inoculations were made with spores from *Avena sativa*, *Calamagrostis canadensis*, or *Festuca*

elator, respectively. A total of 253 inoculations of the species classified as susceptible resulted in 187 positive and 66 negative reactions; 63 developed a few aecia. Ninety of the 187 positive reactions were of normal type.

Those species were classed as resistant which did not function as alternate hosts; that is, those which produced only a maximum development of pycnia, or produced only flecks, or no infections. In this class, 65 of a total of 101 inoculations, or over 64 per cent, were negative. It is obvious that it is increasingly more difficult to secure infection in the resistant than the susceptible class, a condition due probably to the inherent resistance of certain species of *Rhamnus*.

The maximum degree of infection obtained at any time on each species of *Rhamnus* is recorded in Table 3. As the importance of any *Rhamnus* species as an alternate host may depend on its degree of susceptibility under optimum conditions, the maximum degree of infection serves as an index of the possible danger from each particular species. Crown rust obtained from all of the three telial hosts developed aecia on *R. alnifolia*, *R. californica*, *R. californica* var. *tomentella*, *R. cathartica*, *R. crocea*, *R. lanceolata*, *R. rubra*, and *R. smithii*.

TABLE 3.—Maximum infection on *Rhamnus* species, expressed in types of infection 1 to 4, after inoculation with teliospores from three grasses during the period 1919 to 1925, inclusive

Inoculated with teliospores from—	<i>R. alnifolia</i>	<i>R. californica</i>	<i>R. californica tomentella</i>	<i>R. caroliniana</i>	<i>R. cathartica</i>	<i>R. crocea</i>	<i>R. dahurica</i>	<i>R. frangula</i>	<i>R. ilicifolia</i>	<i>R. lanceolata</i>	<i>R. nevadensis</i>	<i>R. pachyphylla</i>	<i>R. pinetorum</i>	<i>R. purshiana</i>	<i>R. rubra</i>	<i>R. smithii</i>	<i>R. tinctoria</i>
<i>Avena sativa</i>	3	3	4	4	4	3	3	2	4	4	* 0	4	4	2	4	4	3
<i>Calamagrostis canadensis</i>	4	4	3	2	3	4	2	2	0	4	---	3	3	2	3	4	2
<i>Festuca elatior</i>	4	4	3	3	4	3	0	0	1	4	---	1	0	0	3	3	0

* 0 = no infection.

Figure 3 represents those species of *Rhamnus* which may function as alternate hosts for the physiologic forms of crown rust on *Avena sativa*, *Calamagrostis canadensis*, or *Festuca elatior*.

OTHER GENERA

Other genera of *Rhamnaceae* and *Eleagnaceae* were studied as alternate hosts of crown rust. The late Ellsworth Bethel, of Denver, Colo., found *Lepargyrea canadensis* and *L. argentea* bearing aecia when growing near gramineous hosts of crown rust. Arthur (1) exposed these two shrubs to teliospores from *Notholcus lanatus* and *Scolochloa festuacea* but obtained no infection.

Lepargyrea canadensis, *L. argentea*, *Ceanothus americanus*, *Berchemia scandens*, and *Zizyphus lycioides* were inoculated in 1921 with teliospores from *Avena sativa*, *Calamagrostis canadensis*, *C. purpurascens*, and *Festuca elatior* (Table 4). On *L. canadensis*, inoculated with teliospores from *C. purpurascens*, a heavy normal infection developed (fig. 4). All other results with this gramineous host were negative. The results on *Lepargyrea* were verified by cultures grown under controlled conditions at Denver, Colo., by I. W. Clokey during the summer of 1922. Unfortunately, no teliospores were available in 1923 with which to inoculate *Ceanothus*, *Berchemia*, and *Zizyphus*.

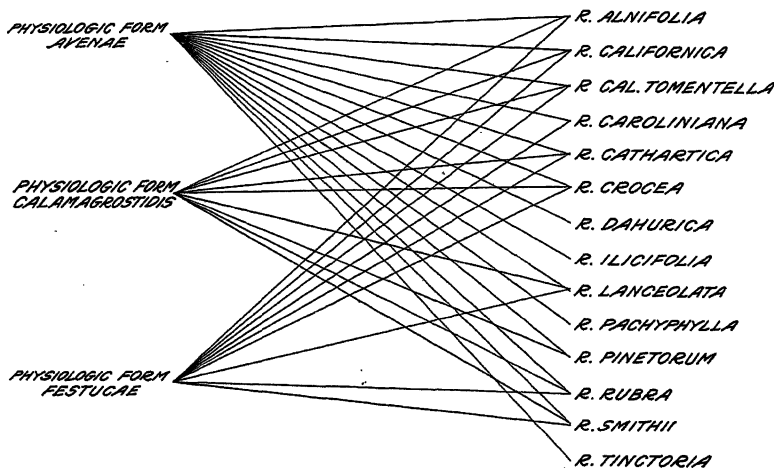


FIG. 3.—Diagrammatic representation of the alternate hosts of three physiologic forms of *Puccinia coronata*

TABLE 4.—Reaction to *Puccinia coronata* of species in genera other than *Rhamnus*

Inoculated with teliospores from—	Species inoculated					
	Number of inoculations on each species	<i>Lepargyrea argentea</i>	<i>Lepargyrea canadensis</i>	<i>Ceanothus americanus</i>	<i>Berchemia scandens</i>	<i>Zizyphus lycioides</i>
<i>Avena sativa</i>	3	0	0	0	+	0
<i>Calamagrostis canadensis</i>	3	0	0	0	0	0
<i>Calamagrostis purpurascens</i>	4	0	+	—	—	—
<i>Festuca elatior</i>	2	0	0	0	0	0

0 = no infection.

+ = infection.

— = not tried.

In July, 1924, aecia were collected on *Lepargyrea canadensis* near Bergen Park, Colo., and aeciospores from these were transferred to *Avena sativa* at Ames, Iowa. Although urediniospores were produced on oats, the results are not conclusive because the aecia were not produced under controlled conditions. *L. argentea* is listed by Arthur (2, p. 313) as an alternate host of crown rust, but the writer was unable to obtain infection through inoculation with teliospores from *A. sativa*, *Calamagrostis canadensis*, *C. purpurascens*, or *Festuca elatior*, at Ames, Iowa.

Berchemia scandens was inoculated on May 5, 1924, with teliospores from *Avena sativa*. By May 19, pyrenia had developed and two days later a few aecia were noted. This experiment was re-

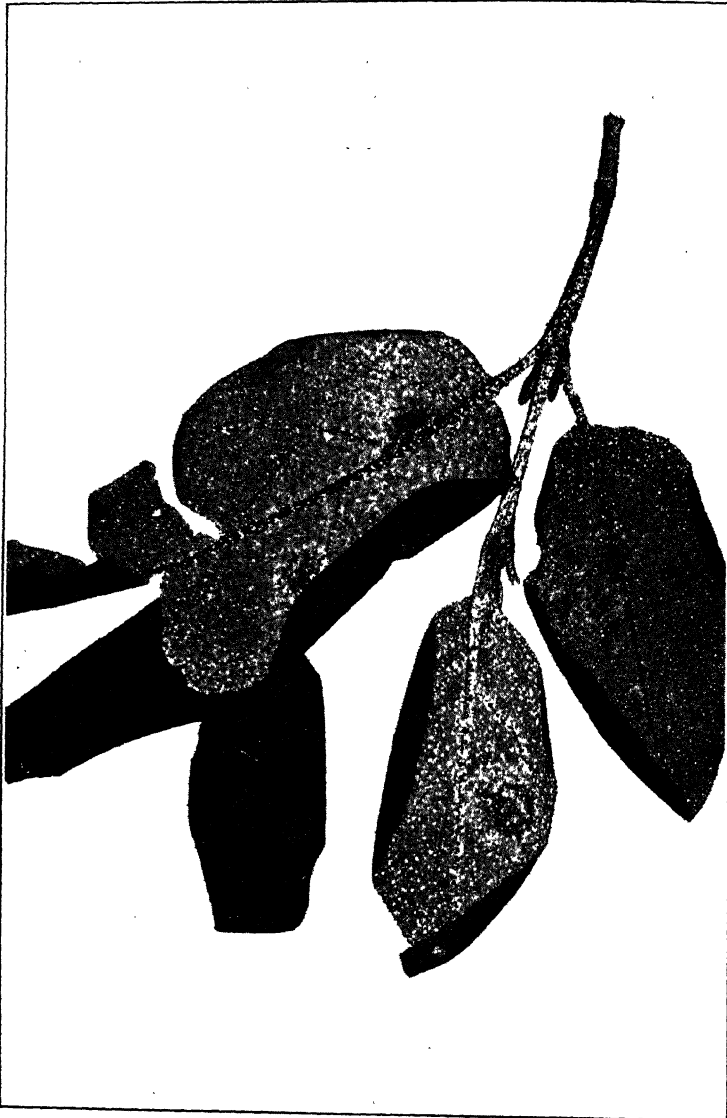


FIG. 4.—*Lepargyrea canadensis* showing aecia after inoculation with teliospores from *Calamagrostis purpurascens*

peated, and four inoculated plants produced a few aecia. *B. scandens* may function as an alternate host for *Puccinia coronata*, although it shows some degree of resistance, as indicated by the small aecia with few cups.

THE EFFECT OF RHAMNUS SPECIES ON PHYSIOLOGIC FORMS OF *P. CORONATA*

As a heteroecious rust like *Puccinia coronata* completes its life cycle on two widely different plants, the question of the possible effect of the alternate host on the biology of the parasite frequently has arisen. Whether the alternate host stimulates or invigorates the fungus in some manner, or changes its biologic response, has not been known. Some light is thrown on the latter phase in this paper.

The range of gramineous hosts of the physiologic forms of crown rust has been previously reported (9). In an earlier report (9) and in the present paper, the reaction of 16 *Rhamnus* species to crown rust is recorded. In order to complete the chain of evidence, the range of gramineous hosts of the aeciospores from *Rhamnus* should be determined.

Inoculating with teliospores from *Avena sativa*, aecial infection was secured on *Rhamnus caroliniana*, *R. cathartica*, *R. dahurica*, *R. ilicifolia*, *R. lanceolata*, *R. pinetorum*, *R. rubra*, and *R. smithii*. *Achyrodes aureum*, *Avena sativa*, *Calamagrostis canadensis*, *Festuca elatior*, and *Notholcus lanatus* were inoculated with aeciospores from each infected species of *Rhamnus*. Aeciospores from all eight *Rhamnus* species caused infection on *Avena sativa*. All results are shown in Table 5.

TABLE 5.—Gramineous host range of *Puccinia coronata* avenae, determined by inoculation with aeciospores from eight *Rhamnus* species

Species	Gramineous hosts				
	<i>Achyrodes aureum</i>	<i>Avena sativa</i>	<i>Calamagrostis canadensis</i>	<i>Festuca elatior</i>	<i>Notholcus lanatus</i>
<i>R. caroliniana</i>	+	+	—	—	+
<i>R. cathartica</i>	—	+	—	+	—
<i>R. dahurica</i>	—	+	—	—	—
<i>R. ilicifolia</i>	—	+	+	+	—
<i>R. lanceolata</i>	+	+	—	—	—
<i>R. pinetorum</i>	—	+	—	—	—
<i>R. rubra</i>	+	+	—	—	—
<i>R. smithii</i>	—	+	—	—	—

+ = infection.

In some cases, the number of aeciospores was limited; hence the negative results of Table 5 are not conclusive. This was true particularly of *Rhamnus dahurica*, *R. ilicifolia*, and *R. pinetorum*. Inoculum from *R. caroliniana*, *R. cathartica*, *R. lanceolata*, *R. pinetorum*, *R. rubra*, and *R. smithii* produced urediniospores on *Notholcus lanatus*. Urediniospores were produced on *Calamagrostis canadensis* inoculated with aeciospores from *R. ilicifolia* and *R. lanceolata*. *Achyrodes aureum* has been shown to be a host for five physiologic forms of *Puccinia coronata*, two physiologic forms of *P. graminis* and *P. dispersa*, *P. montanensis*, and *P. poarum*. Urediniospores were produced on this grass through inoculation with aeciospores from *R. caroliniana*, *R. cathartica*, *R. lanceolata*, and *R. rubra*.

Rhamnus lanceolata, which had been inoculated with teliospores from *Calamagrostis canadensis*, produced abundant urediniospores on *Avena sativa* in nine trials conducted over a period of three years.

CORRELATION OF LABORATORY AND FIELD RESULTS

Although *Rhamnus* species may become infected with crown rust under greenhouse conditions, the importance of the alternate hosts of this rust depends on such factors as prevalence and distribution of the species, the quantity of infection, together with the methods of infection, rate of dissemination of their aeciospores, and the resulting damage to the oat crop.

RHAMNUS CATHARTICA IN THE UPPER MISSISSIPPI VALLEY

Through the cooperation of the State leaders of barberry eradication and their field assistants with the plant pathology section of the Iowa Experiment Station, a preliminary survey for the locations of common buckthorn (*Rhamnus cathartica*) was made. In representative areas, comprising less than one-twentieth of the total area in each of the eight upper Mississippi Valley States, a total of 1,021 plantings of *R. cathartica* was found during 1923 and 1924. Of these plantings, 282 were rural and 739 urban. They contained 93,041 counted bushes besides 44,395 feet of hedge where the bushes were not counted. Many wild areas of escaped bushes found in Illinois, Wisconsin, and Montana were not included in this count, for the bushes were too numerous to estimate.

It readily can be seen that with this tremendously large number of shrubs in a relatively small area, a serious, though local, annual damage to the oat crop may result if these shrubs become infected. Since the aecial infection affecting the oat crop could function only a short time in the spring, the percentage of bushes infected was tabulated for the months of May and June, 1923. Nearly 80 per cent of all bushes reported during this period were infected, while those reported from the farmsteads were uniformly infected. In 1924, about 50 per cent of the bushes located during this period were infected. For the past nine years, under Iowa conditions, aecial infection of *Puccinia coronata* developed on *Rhamnus cathartica* and *R. lanceolata* before uredinia were present on *Avena sativa* (Table 6).

TABLE 6.—Time of appearance of crown-rust uredinia on *Avena sativa* and aecia on *Rhamnus cathartica* and *R. lanceolata* in Iowa for the period 1916 to 1924, inclusive

Stage in life cycle	Host	Year				
		1916	1917	1918	1919	1920
Aecial.....	<i>Rhamnus cathartica</i>	May 7	June 30	Apr. 30	May 26	May 15
Aecial.....	<i>Rhamnus lanceolata</i>	May 22	June 6	June 6	May 15	May 20
Uredinal.....	<i>Avena sativa</i>	June 2	July 6	July 6	June 6	June 12

Stage in life cycle	Host	Year			
		1921	1922	1923	1924
Aecial.....	<i>Rhamnus cathartica</i>	May 16	May 14	May 21	May 28
Aecial.....	<i>Rhamnus lanceolata</i>	May 16	May 21	May 23	May 7
Uredinal.....	<i>Avena sativa</i>	May 22	June 14	June 16	June 16

The earliest appearance of aecia during this period was found on *Rhamnus cathartica* on April 30, and the latest on June 30. The time of the appearance of infection depends somewhat on climatic factors, especially moisture and temperature. *R. lanceolata* varied in time of observed infection from May 7 to June 6. Uredinia usually appear on oats about seven days after visible outbreak of the disease on the alternate host. However, this period was increased greatly in 1918* by the hot, dry weather intervening between aecial and uredinial infection.

In order to prove further that buckthorn was responsible for the initial infection in Iowa, a detailed survey of the State was made during May and June, 1923 and 1924. If wind-blown spores from the south started the infection in Iowa, a more or less uniform rust spread should have appeared first across the southern part of the State. However, during this time, many local areas or concentric zones of crown-rust infection of oats were observed scattered over the State previous to a general infection. Either *Rhamnus cathartica* or *R. lanceolata* was found near the center of each of these areas. The degree of infection varied from a trace on the outer edge of these concentric zones to 30 per cent near the center. These local infection centers appeared simultaneously in northern and southern Iowa. The areas were small at first, remained constant for 7 to 10 days, and then expanded rapidly. This probably was due partly to the limited area over which aeciospores cause infection (4). It is probable that infection from rural plantings of *Rhamnus* is more likely to spread than that from city plantings. This belief is supported by the fact that the centers of all these local infection areas were on farmsteads.

A detailed study of the effect of crown rust on yield was made at Winthrop in Buchanan County, Iowa, where the initial infection was a hedge of *Rhamnus cathartica* adjoining an oat field. The oats were not cut, as many of the plants failed to head. Oats in adjoining sections yielded 10 bushels per acre, while the average yield of more remote sections of the same township was 50 bushels per acre.

RHAMNUS FRANGULA

As previously stated, the maximum infection on *Rhamnus frangula* under controlled conditions gave only pycnia production in 4 out of 48 trials. It seemed desirable to ascertain whether the species maintained this relative resistance under field conditions. During 1923 and 1924, ten plantings or thickets of *R. frangula* in the New England States, ranging from a few to more than 2,000 bushes were inspected for aecial infection. In four of these cases, where *R. frangula* and *R. cathartica* were growing side by side, and often interwoven, aecial infection was found abundantly on *R. cathartica* but was absent on *R. frangula*. Likewise no aecia were found on the other six plantings of *R. frangula*. *R. frangula* bushes, growing on the campus at Ames, Iowa, have produced no aecia during the past eight years of observation, while adjoining bushes of *R. cathartica* were infected annually.

RHAMNUS PURSHIANA

Under controlled conditions, *Rhamnus purshiana*, like *R. frangula*, proved resistant to crown rust. It is a well-known fact that this species produces abundant aecia under natural conditions, although

it remains to be discovered just what, if any, relationship this aecial infection bears to *Puccinia coronata* on *Avena sativa*.

In June, 1922, at New Amsterdam, B. C., the late Ellsworth Bethel succeeded in infecting *Calamagrostis* sp. by inoculating with aeciospores taken from *Rhamnus purshiana* grown in the open.

DISCUSSION

In Europe, crown rust (*Puccinia coronata*) has been divided by Klebahn (7) into two species, *P. coronata* and *P. coronifera*. Eriksson (5) and Mühlethaler (10) are in agreement with Klebahn that *Rhamnus frangula* acts as a differential host for *P. coronata* and *P. coronifera*. Eriksson (5) recognizes Barclay's *P. coronata* var. *himalensis* as still further distinct, since it produces aecia on *R. dahurica*. Dietel makes a valid species of this form, and Mühlethaler (11) recognizes it, but suggests that it may be *P. coronifera*. These investigators, then, have employed the several species of *Rhamnus* as indicators of different species of rust. In other words, strong emphasis has been placed on the physiologic reaction of the alternate host. Treboux (12), on the other hand, fails to find the marked differential reaction of *Rhamnus* species reported by Klebahn (7), Eriksson (5) and Mühlethaler (11).

Still further, here in America, Melhus, Dietz, and Willey (9) have presented infection experiments suggesting lack of marked differential action of *Rhamnus cathartica* and *R. frangula*. They found that teliospores from *Avena sativa* produced pycnia on *R. frangula* and that the 10 species considered by them showed different degrees of susceptibility ranging from flecking to the normal production of aecia. This varied host behavior led these authors to question the wisdom of founding a species of *Puccinia* on the basis of its alternate-host reaction.

In these further studies, comprising inoculation trials extending over four years and involving 16 species of *Rhamnus*, a wealth of additional data has been obtained bearing directly on the reaction of *Rhamnus* species to crown rust. It has been shown again that *R. frangula* is not immune from infection when inoculated with teliospores from *Avena sativa* and *Calamagrostis canadensis*. The development of pycnia was obtained on four different occasions. Infection is very dependent on the state of development of the leaf tissues and on the environmental conditions. It is quite conceivable that, if infection can be induced in the greenhouse, it also may be found under certain conditions in the open. It should be noted, however, that *R. frangula* is much more resistant than *R. cathartica* and *R. lanceolata*, although neither Klebahn (7), Eriksson (5), nor Mühlethaler (10, 11) noted any variation in the degree of susceptibility of the two first named species.

Up to the present writing, teliospores of *Puccinia coronata* from *Avena sativa*, *Calamagrostis canadensis*, *Festuca elatior*, and *Notholcus lanatus* have been applied as inoculum to species of *Rhamnus*. Infection on *R. frangula* has resulted only through teliospores from *A. sativa* and *C. canadensis*. None has developed through teliospores from *F. elatior* and *N. lanatus*, probably because of the fewer trials with spores from the two last mentioned hosts and the added difficulty of securing viable teliospores from *Festuca* and *Notholcus*.

Further evidence showing that the alternate-host range of the American crown rust differs from that found in Europe is apparent in the reaction of *Rhamnus dahurica*. Barclay describes a variety of *Puccinia coronata* as *P. coronata* var. *himalensis* on *Agrostis himalensis*. Later, Eriksson transferred this variety to *R. dahurica* and subsequently Dietel raised Barclay's variety to specific rank, naming it *P. himalensis*. Mühlethaler subsequently recognized this species, but suggested that it might be synonymous with *P. coronifera* Kleb. As shown in Table 1, this method of differentiation is questionable where teliospores from *Avena sativa* produced aecia on *R. dahurica*, which, in turn, was able to produce urediniospores on *A. sativa* (Table 5). These data show that *R. dahurica* can not act as a differential host for *P. himalensis* (Barcl.) Diet., since teliospores from *A. sativa* also can produce aecia on this alternate host.

Another very significant point bearing on the reaction of *Rhamnus* species to infection is the preference manifested by crown rust taken from different gramineous hosts. As shown in Table 1, *Puccinia coronata* from *Avena sativa* infects most readily *R. cathartica* and *R. lanceolata*. *R. alnifolia* is most susceptible to the *Calamagrostis* form taken from *C. canadensis*. However, in each case, different degrees of infection were obtained on at least 15 species. At the same time, *R. alnifolia* has consistently shown resistance to the *A. sativa* form. In the case of *R. caroliniana*, the differential reaction of the two sources of crown rust is even more marked. Teliospores from *A. sativa* gave normal infection, while those from *C. canadensis* showed only pycnial development.

Although the reaction of crown rust from all the gramineous hosts has not been fully studied up to the present writing, the data available indicate clearly that certain species of *Rhamnus* are markedly resistant to some and susceptible to others. In other words, a physiologic form has its alternate-host range as well as its gramineous host range. Clearly, then, when the reaction of additional physiologic forms has been studied, still further overlapping and extension of alternate-host ranges should be discovered. As shown earlier, crown rust from *Avena sativa* was transferred for the first time to *Berchemia scandens*, one of the *Rhamnaceae*.

An additional extension of the alternate-host range of crown rust developed when infection was obtained on *Lepargyrea canadensis* of the family *Eleagnaceae* through inoculation with teliospores from *Calamagrostis purpurascens*. It is not yet clearly understood just what alternate-host range and particular specialization that form of crown rust may have which produces abundant aecia on *Rhamnus purshiana*, a species indigenous to British Columbia, California, Oregon, Washington, and Idaho. The data here presented show the alternate-host specialization and the marked development of resistance and susceptibility of the physiologic forms *Avenae* and *Calamagrostidis*. These, combined with the extension of the alternate-host range beyond the genus *Rhamnus* and, in fact, outside the family *Rhamnaceae*, allow no alternative but to consider the crown-rust organism as one species with a wide alternate-host range. Crown rust, then, must remain as *Puccinia coronata* Corda.

Another point of much importance in connection with the rust reaction of *Rhamnus* is the influence exerted by different species of the host on the stability of the physiologic forms of the rust.

Among the European students of crown rust, Treboux alone, as noted earlier, presented results at variance with those of other workers. He obtained infection of grasses directly through aeciospores, while most of the other European investigators have sought to infect *Rhamnus* through teliospores of various grasses. Treboux (12, 13) produced urediniospores on *Avena sativa* through inoculation with aeciospores from *R. frangula*. He also found that the aeciospores from *R. cathartica* infected *Agrostis stolonifera*, *Calamagrostis arundinacea*, and *Phalaris arundinacea*, all of which are listed as hosts of *Puccinia coronata* by Klebahn and Mühlethaler. On the other hand, in localities where only *R. frangula* occurs, two of the hosts of *P. coronifera*, namely, *A. alba* and *Poa pratensis*, were infected with *P. coronata*. Treboux took this to mean that specialization as reported by Klebahn and others probably did not exist. However, to the writer, Treboux's results, in connection with data here presented, suggest that the stability of the physiologic forms was changed through their culture on the *Rhamnus* hosts.

As shown in Table 5, teliospores from *Avena sativa* produced infection on eight species of *Rhamnus*. The aeciospores from each of these, in turn, caused abundant infection on *Notholcus lanatus* and *A. sativa*. This result is directly opposed to ordinary experience, for the form of crown rust on *N. lanatus* is highly specialized (9), only occasionally producing a subnormal infection on *A. sativa* when urediniospores are used as inoculum. Again, teliospores from *Calamagrostis canadensis* caused infection on *R. lanceolata*, these aeciospores producing urediniospores on *A. sativa* many times during a period of three years. It has been shown in an earlier paper (9) that the urediniospores on *C. canadensis* can only occasionally produce a subnormal infection on *A. sativa* and vice versa. On the other hand, teliospores from *A. sativa* produced aeciospores on *R. lanceolata* which produced urediniospores on *C. canadensis* (Table 5). This suggests that the physiologic-form relationship as expressed by the gramineous hosts may be altered by the *Rhamnus* host.

SUMMARY

The division of *Puccinia coronata* Corda into two species, as has been done by Klebahn, in Europe, is not justified in America.

The fact that *Rhamnus dahurica* produces aecia through teliospores from *Avena sativa* prevents this species of *Rhamnus* from functioning as a differential host for *Puccinia coronifera* Kleb. and *P. himalensis* (Barcl.) Diet., although Eriksson and Mühlethaler in Europe have claimed that it could so serve.

The alternate hosts of *Puccinia coronata* are not restricted to the genus *Rhamnus* or even to the family Rhamnaceae.

Thirteen species and one variety of *Rhamnus*, indigenous to the United States, and three species native to Europe, were investigated as possible alternate hosts of crown rust, from 1 to 28 times each, making a total of 364 trials. When *Rhamnus californica* var. *tomentella*, *R. caroliniana*, *R. cathartica*, *R. ilicifolia*, *R. lanceolata*, *R. pachyphylla*, *R. pinetorum*, *R. rubra*, and *R. smithii* were inoculated with teliospores from *Avena sativa*, the resulting infection was normal in at least one trial. *R. alnifolia*, *R. californica*, *R. crocea*, *R. dahurica*, and *R. tinctoria* also are susceptible, and may function

as alternate hosts of the Avenae form of *P. coronata*. *Rhamnus frangula*, *R. nevadensis*, and *R. purshiana* reacted with marked resistance to the physiologic form on *Avena sativa*.

Rhamnus alnifolia, *R. californica*, *R. crocea*, *R. lanceolata*, and *R. smithii* developed normal infection when inoculated with teliospores from *Calamagrostis canadensis*. It also was found that *R. californica* var. *tomentella*, *R. cathartica*, *R. pinetorum*, and *R. rubra* may function as alternate hosts of this physiologic form. The other eight species showed marked resistance.

Teliospores from *Festuca elatior* caused normal infection on *Rhamnus alnifolia*, *R. californica*, *R. cathartica*, and *R. lanceolata*. *R. californica* var. *tomentella*, *R. caroliniana*, *R. crocea*, *R. rubra*, and *R. smithii* produced a few aecia. Twelve trials on the seven remaining species showed that *R. ilicifolia* produced pycnia and *R. pachyphylla* developed flecks only.

Teliospores from *Notholcus lanatus* produced pycnia on *Rhamnus lanceolata*.

Ceanothus americanus, *Lepargyrea argentea*, and *Zizyphus lycioides* did not respond as alternate hosts of crown rust. *Berchemia scandens* was infected with teliospores from *Avena sativa*. *Lepargyrea canadensis* produced aecia when exposed to infection with teliospores from *Calamagrostis purpurascens*.

Rhamnus species, functioning as alternate hosts, may alter the physiologic response of specialized forms.

A total of 1,021 plantings of *Rhamnus cathartica* were found in the upper Mississippi Valley. Of these, 282 were rural and 739 urban, with a total of 93,041 bushes, besides 44,395 feet of buckthorn hedge of uncounted bushes. In addition, many areas of escaped plants were located in Illinois, Montana, and Wisconsin.

During the past nine years in Iowa, aecial infection has appeared on *Rhamnus cathartica* and *R. lanceolata* previous to the development of uredinia on *Avena sativa*. These two species of *Rhamnus* have been instrumental in starting local and general epidemics in Iowa within the past two years.

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THE INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS¹

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INTRODUCTION

The effect of temperature upon the development of the disease of cabbage caused by the vascular parasite *Fusarium conglutinans* Wollenw. has been studied by Gilman (5)² and Tisdale (14). Gilman found that this disease appears in its most destructive form in the fields under conditions of hot, dry weather, whereas it develops but slightly during moist, cool weather. Tisdale determined more exactly the relation of soil temperature to the development of the disease in cabbage seedlings. He found that cabbage yellows develops in seedlings growing in "sick" soil at soil temperatures ranging from 17° to 35° C., the optimum for rapid development of the disease being from 26° to 29°. This optimum temperature coincides with the optimum for vegetative growth of the fungus in pure culture and is distinctly above the optimum for growth of cabbage seedlings, which Tisdale found to be about 20°. He found also that yellows occurs at any percentage of soil moisture at which the cabbage seedlings grow, but that it develops most rapidly and destructively at about 19 per cent of the dry weight. This soil moisture is also the most favorable for the normal growth of the host plant. Tisdale found that seedlings of the resistant Wisconsin Hollander and the susceptible Commercial Hollander strains up to the age of 20 days were almost equally susceptible to yellows. However, as the age of the plants increased resistance became more pronounced in the resistant strain. He used only plants of Wisconsin Hollander and Commercial Hollander.

The purpose of the study herein reported was sixfold: (1) To determine the range of soil temperatures at which yellows occurs in the young seedlings of Wisconsin All Seasons and Commercial All Seasons strains; (2) to determine the effect of various soil temperatures upon the resistant quality of the selected cabbage strains; (3) to study further the range of soil temperatures for the development of yellows in both resistant and susceptible cabbage strains; (4) to determine the difference in the development of the disease in cabbage plants grown at high and at low temperatures before being transplanted to diseased soil; (5) to study the effect of different combinations of air and soil temperatures on yellows development; and (6) to study further the influence of soil moisture upon the occurrence of yellows.

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² Reference is made by number (italic) to "Literature cited," p. 991.

METHODS AND EQUIPMENT

The apparatus employed for controlling the soil temperature in these experiments was what is known as the Wisconsin soil-temperature tank. Since this apparatus has been described by Jones (9) and a modified form by Dickson (3), further description is unnecessary. The temperatures below 20° C. were regulated by running in small quantities of cold water as often as required to keep the temperature of the water in the tank fairly constant. The higher temperatures were maintained by the use of electric lamps controlled by thermostats. With careful attention at regular intervals, the temperatures seldom varied more than 1° or 2° from those desired, and then for only a short time.

Most of the experiments were conducted in a greenhouse room with the temperature at about 20° C. This was fairly constant except during the spring months beginning late in March, when the sun caused a rise in temperature which could not be entirely eliminated by means of the blower. Other experiments were conducted under different air temperatures, which will be discussed separately with the various experiments.

RECEPTACLES

The culture cans were made of galvanized sheet iron, cylindrical, about 6 inches in diameter and 10 inches deep. They were prepared for use by first being brought to an even weight by the addition of coarse gravel to the lighter cans. A 2-inch clay pot with a one-half-inch hole in the bottom was upturned in the bottom of the can and the soil put in up to within about 1 $\frac{1}{2}$ inches of the top, an equal weight of soil being placed in each can. The water was supplied through a test tube with a broken bottom, which was placed in the center of the receptacle with the lower end connected with the hole in the bottom of the flowerpot. After the cans were filled with soil they were so placed in the tanks that the surface of the soil was about level with the water in the tank.

SOIL

The naturally infested soil used in all the experiments was obtained from a "cabbage sick" field in Kenosha County, Wis. Before being used, the soil was screened and uniformly mixed with a virgin clay loam in the ratio of 5 parts of the infested soil to 1 of virgin soil.

The moisture content of the soil in all the temperature experiments, with the exceptions mentioned later, was kept at about 20 per cent of the dry weight or 40 per cent of the moisture-holding capacity, as determined by the 10-inch soil cylinder method. Tisdale (14) found that with favorable temperature the yellows disease develops most rapidly and destructively in soil with about 19 per cent moisture on the dry-weight basis, or 40 per cent on the wet basis. This moisture content was used in order to give the plants as severe a test as possible. The receptacles were weighed at regular intervals and water added to keep the soil at about the moisture content desired.

SEED

The Wisconsin All Seasons seed used in all the experiments unless otherwise specified was from a lot of seed grown in the Puget Sound section of Washington in 1921. This strain of cabbage had been

proved by Jones et al. (10) to be highly resistant to yellows under field conditions. The Commercial All Seasons seed was taken from a single lot grown on Long Island, N. Y., in 1921 and obtained from a seed company in Madison, Wis. Two lots of Wisconsin Hollander seed were also used. Lot 1 was grown in 1921 by W. A. Walker at Racine, Wis., and lot 2 was grown in Washington State in 1923 from the seed of lot 1. The Commercial Hollander seed was grown in Denmark in 1921.

RELATIVE SUSCEPTIBILITY TO YELLOWS OF "RESISTANT" AND "SUSCEPTIBLE" STRAINS OF CABBAGE SEEDLINGS

Seed of Wisconsin All Seasons and Commercial All Seasons strains were planted in naturally infested soil in the receptacles previously described and placed in tanks at temperatures of 16°, 20°, 24°, 28°, and 32° C., four cans of each variety at each temperature. When the seedlings had emerged from the soil they were thinned so as to leave 10 plants to each receptacle.

Data were taken 10, 13, 17, and 20 days after planting. Percentages of yellows, as given in Table 1, include the plants which showed the disease in incipient stages as well as those which died of it. The All Seasons strains tested reacted to yellows in a way similar to those tested by Tisdale, who found that there was little difference in percentage of disease between resistant and susceptible Hollander strains up to the age of 29 days. However, the disease was somewhat slower in developing in the plants of the resistant strains, and the final percentages of disease were somewhat lower in these than in plants of the susceptible strain.

TABLE 1.—*Percentage of cabbage seedlings growing in naturally infested soil which developed yellows at different soil temperatures*

[Air temperature from 20° to 22° C.]

Soil temperature °C.	Number of plants of each variety	Percentage of Commercial All Seasons diseased after—				Percentage of Wisconsin All Seasons diseased after—			
		10 days	13 days	17 days	20 days	10 days	13 days	17 days	20 days
16.....	40	0	0	0	0	0	0	0	0
20.....	40	0	0	12	32	0	0	15	22
24.....	40	8	42	54	74	8	35	50	53
28.....	40	25	60	65	82	10	32	45	80
32.....	40	22	62	76	70	20	38	50	55

It has been observed by Edgerton and Moreland (4) that tomato wilt develops more slowly in a resistant strain and that the plants do not die as rapidly as those of the susceptible strains. According to these investigators—

all varieties, even the most resistant, when grown under the most favorable conditions, show a rather heavy infection with the wilt disease. Susceptible varieties, however, take the wilt earlier, die quicker, and in most cases show a higher percentage disease than resistant ones.

Somewhat the same condition seems to obtain in the strains of cabbage so far produced, except one recently developed by Walker et al. (17), which appears to be practically immune to yellows. Under field conditions there may be a high percentage of yellows in

some of the resistant strains, but most of these are able to withstand or resist the attack of the fungus to a greater degree than the susceptible strains. The difference between the resistant and susceptible strains seems to lie in the ability of the former to prevent the development of the fungus after it has once gained access to the tissues. There is evidently a sort of balance reached between the host and parasite in the case of the resistant strains under ordinary field conditions. When the soil temperature goes up and the soil moisture is reduced, this balance is upset and the fungus makes further inroads on the tissues of the plant. If these conditions prevail for a sufficient length of time the plants may be killed; but in general they do not persist long enough to cause the death of many plants of the resistant strains. With the advent of cooler weather the earlier balance between the host and the parasite is restored and the cabbage plants again become "resistant" and develop normally.

In January, 1923, plants of the Wisconsin Hollander and Commercial Hollander strains, grown in virgin soil for 55 days at an air temperature of from 14° to 18° C., were transplanted to receptacles containing naturally infested soil. Four plants of each sort were placed in separate receptacles, one can of each kind in each of five tanks maintained at temperatures of 12°, 18°, 24°, 28°, and 32°, respectively. Observations were made 13, 21, and 33 days after transplanting. The results, shown in Table 2, indicate that within 13 days after they were placed in diseased soil at temperatures of 24°, 28°, and 32°, half or more of the plants of the resistant Wisconsin Hollander showed symptoms of disease. Two of this strain in the 24° tank were diseased at the end of the thirteenth day after transplanting, and at the end of 33 days all four were diseased and two were dead. In the case of the susceptible Commercial Hollander, however, three-fourths of the plants in the 24° tank were dead by the thirteenth day and all were dead by the twenty-first day. At the conclusion of the experiment it was noted that all of the susceptible strain were dead in the 24°, 28°, and 32° tanks, whereas 50 per cent of the resistant strain were still alive in the 24° tank, 75 per cent in the 28° tank, and 75 per cent in the 32° tank.

TABLE 2.—Variations in susceptibility among cabbage seedlings of Commercial Hollander and Wisconsin Hollander strains, transplanted from healthy to naturally infested soil, and grown at different soil temperatures

Soil temperature (°C.)	Num-ber of plants tested	Commercial Hollander					Wisconsin Hollander				
		Number of plants diseased after—			Per cent diseased at end of experiment	Per cent dead at end of experiment	Number of plants diseased after—			Per cent diseased at end of experiment	Per cent dead at end of experiment
		13 days	21 days	33 days			13 days	21 days	33 days		
12.....	4	0	0	(a)	0	0	0	0	(a)	0	0
18.....	4	0	0	(a)	0	0	0	0	(a)	0	0
24.....	4	3	4	4	100	100	2	4	4	100	50
28.....	4	3	4	4	100	100	3	3	3	75	25
32.....	4	3	4	4	100	100	2	3	3	75	25

^a Plants in diseased soil at 12° and 18° C., were removed 21 days after transplanting to tank at 28°. The Commercial Hollander plants were all dead within 10 days, and 6 of the 8 Wisconsin Hollander plants were diseased at the end of 24 days.

^b One plant showing severe yellows symptoms put out new roots near the surface of the soil and survived the attack of the fungus.

In Table 3 are summarized the data from several soil temperature experiments with plants grown under different conditions. The results are rather difficult to interpret because of the great variation in the percentage of disease among plants of the different lots. These data certainly confirm some things, however, that have been noted before, namely, that there is a wide variation in the percentage of disease, its severity, and the length of the incubation period among plants of both resistant and susceptible strains. The slight differences in the size and vigor of the plants before they were placed in diseased soil might have been caused by a difference in environmental factors such as water supply and sunlight, or by rapidity of growth, and this initial strength or weakness might account for some variations in susceptibility among the seedlings to the yellows disease. In addition to these factors, there must also be the factor of heterozygosity in the genetic composition of the plants. Unless resistance is inherited as a simple Mendelian character it is quite likely that the average lot of cabbage seed is heterozygous for the factors for resistance, since the cabbage plant is normally cross fertilized. Furthermore, in the selection of resistant strains there has been no attempt to acquire homozygosity by selfing of plants through a series of generations, simple mass selection having been made in all cases until the very recent work of Walker (16).

In general, it seems that vigorous plants are most subject to the attack of the yellows parasite. In some cases it was found that plants of the commercial strains, which are usually highly susceptible to yellows, when grown under conditions of low soil moisture and high soil temperature, during periods of low light intensity, often remained in diseased soil for from two to four weeks without showing a very high percentage of disease. These plants grew very slowly and the leaves became somewhat leathery in texture. Especially was this noticeable during midwinter when there was often no direct sunlight for several days in succession.

In the next experiment, which was begun on February 8 and ended March 9, seven soil temperatures were used beginning at 15° C., and increasing in increments of 3° to 33°. Wisconsin All Seasons and Commercial All Seasons plants were grown in healthy soil at an air temperature of about 20° for 55 days before being removed to diseased soil. Three plants were placed in each receptacle, and five receptacles containing resistant plants and three containing susceptible plants were used. The receptacles were kept at 20° air temperature for 24 hours before they were placed in the tanks.

Beginning 9 days after transplanting, observations were made at intervals until the plants had been in diseased soil for 30 days. The results are shown in Table 4 and Figures 1, 2, and 3. It will be noted from an examination of Figure 2 that beginning at a soil temperature of 24° C. and continuing to 33° there is very little difference in resistance between the plants of the resistant strain and those of the susceptible. Even less disease occurred in the latter strain at 33° than in the resistant strain, no disease being evident in any of the 9 plants of the commercial strain, whereas 3 of the 15 plants of the resistant strain showed yellows, 2 of them having succumbed to the disease. At 21°, however, there was a striking difference in the percentage of diseased plants in the resistant and susceptible strains,

33 per cent showing disease in the Wisconsin All Seasons at this temperature as compared with 89 per cent in the Commercial All Seasons plants.

TABLE 3.—*Effect of high soil temperature and low soil moisture upon the development of yellows in cabbage plants—summary of four experiments*

Variety	Date of transplanting	Age of plants in days	Soil temperature before transplanting	Air temperature	Soil temperature after transplanting	Number of days in sick soil	Number of plants	Per cent diseased	Per cent dead	Remarks
Wisconsin: All Seasons.	Jan. 22, 1923	60	° C. 12-15	° C. 25-28	° C. 20-23	60	50	* 27	20	In flats
Wisconsin: Hollander.	Jan. 26, 1923	55	14-16	20-23	24	23	4	100	75	In tanks.
Commercial Hollander.do.....	55	14-16	20-23	32	23	4	100	50	
Wisconsin All Seasons.	Mar. 9, 1923	82	16-18	28	24	23	12	100	80	Do.
Commercial All Seasons.do.....	82	16-18	28	28	45	10	90	90	Do.
Wisconsin All Seasons.	Apr. 4, 1923	90	15-22	28	28	23	36	15	11	Do.
Wisconsin All Seasons.	Apr. 28, 1923	60	20-22	24	24	33	48	50	32	Do.
Commercial All Seasons.do.....	60	20-22	24	24	33	16	100	100	Do.

* Plants in flats of diseased soil where soil temperature and soil moisture were not under control

TABLE 4.—*Effect of temperature on the development of yellows in Wisconsin All Seasons and Commercial All Seasons cabbage plants*

WISCONSIN ALL SEASONS

Soil temperature (°C.)	Number of plants of each variety	Percentage of plants diseased or dead after—															
		9 days		12 days		15 days		18 days		21 days		24 days		27 days		30 days	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	15	0	0	0	0	0	0	0	0	0	0	13	0	33	0	33	0
24	15	0	0	0	0	13	0	27	0	40	0	47	0	59	27	66	27
27	15	7	0	27	0	33	0	46	6	60	20	70	52	70	70	77	70
30	15	0	0	13	0	27	0	52	7	60	40	67	52	70	59	70	59
33	15	0	0	13	0	13	0	13	0	20	0	20	0	20	13	20	13

COMMERCIAL ALL SEASONS

15	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	9	0	0	0	0	0	0	0	0	0	0	0	0	11	0	22	0
21	9	11	0	22	0	22	0	22	0	33	0	44	0	78	33	89	33
24	9	11	0	33	0	44	0	67	11	67	22	67	67	67	67	67	67
27	9	11	0	22	0	22	0	33	11	78	33	78	44	78	55	78	55
30	9	11	0	33	0	33	0	44	22	78	22	89	66	89	66	89	66
33	9	0	0	0	0	0	0	0	0	11	0	11	0	11	0	11	0

* One plant slightly diseased.

The curves for plants killed by yellows, shown in Figure 1, parallel those for percentage of disease quite closely up to 24° C., but a difference in percentage of plants killed by yellows at 24° is observed

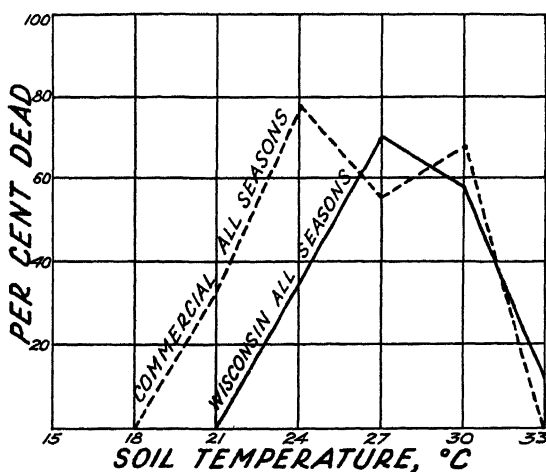


FIG. 1.—Comparison of the death rate from yellows of Commercial All Seasons and Wisconsin All Seasons plants grown for 30 days in naturally infested soil. (See Table 4)

comparable to the percentage of disease shown in Figure 2 at 21°. Although at 27° a higher percentage of plants of the resistant Wisconsin strain were killed than of the more susceptible commercial strain, the curve for death of the latter has two maxima, one at 24°

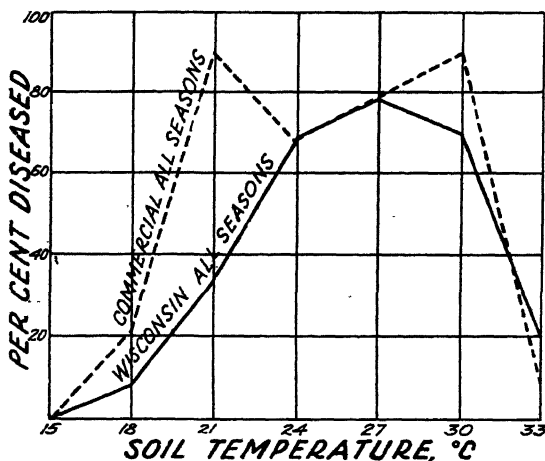


FIG. 2.—Comparison of the development of yellows in Commercial All Seasons and Wisconsin All Seasons plants grown 30 days in naturally infested soil. (See Table 4)

and the other at 30°. These results differ from those obtained by Tisdale (14), who found 29° to be the optimum temperature for yellows development in the Wisconsin Hollander variety and 26° for plants of the Commercial Hollander. The curve would probably be straightened somewhat if the average of several experiments was used.

The incubation period of the disease varied a good deal in the two strains with the temperature of the soil. For example, as shown in Figure 3, B, at a soil temperature of 21° the maximum percentage of disease was reached in the Wisconsin All Seasons plants 27 days after transplantation to diseased soil, whereas the maximum among the susceptible plants was not reached until the thirtieth day. The

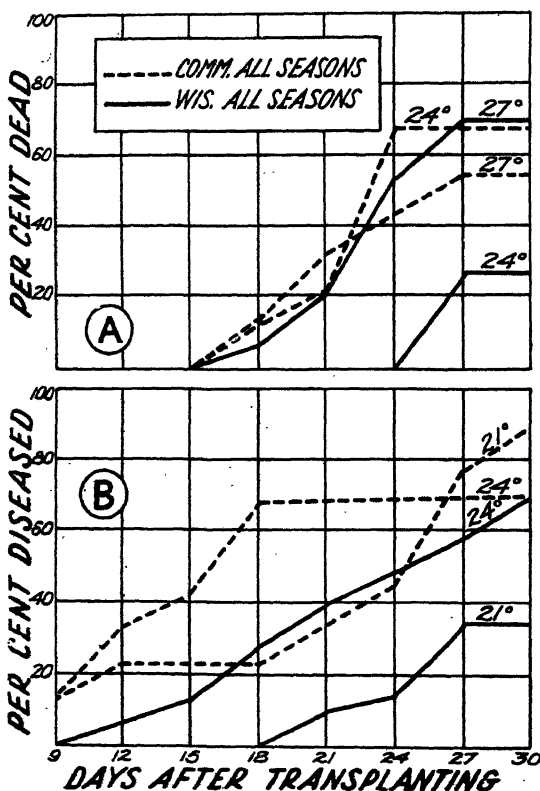


FIG. 3.—Comparison of the rate at which yellows developed in Commercial All Seasons and Wisconsin All Seasons plants grown in naturally infested soil at different temperatures. (See Table 4)

in the commercial strains. And this is more striking at 21° and 24°, which represent most nearly the extreme of the mean soil temperature ordinarily encountered in the field.

RELATION OF SOIL AND AIR TEMPERATURES TO THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS

Clayton (1), in his study of the effect of soil temperature on the development of tomato wilt, found that the optimum temperature for growth of the disease was about 29° C. Edgerton and Moreland (4) found that tomato wilt developed more slowly if the temperature was much below 29°. Clayton (2) grew tomato plants in soil at temperatures of 17°, 27°, and 33° at each of three air temperatures, namely, 17°, 27°, and 33°, and found that of the nine combinations

of air and soil temperatures only two produced wilt to any appreciable extent. These were (1) soil and air temperature both 27° , and (2) soil 27° and air 33° . When the plants were kept at a high soil temperature and a comparatively low air temperature the vascular bundles of the plants became discolored from the ground line downward, showing that the parasite had penetrated the lower parts of the plant, but no symptoms of wilt appeared above the ground line. Plants from a soil and air temperature of 33° showed only very faint traces of wilt and slight discoloration of the vascular bundles. These results seem to indicate that the tomato-wilt organism is unable to penetrate to any great extent those parts of the plant which are surrounded by cool air, that is, air at about 17° .

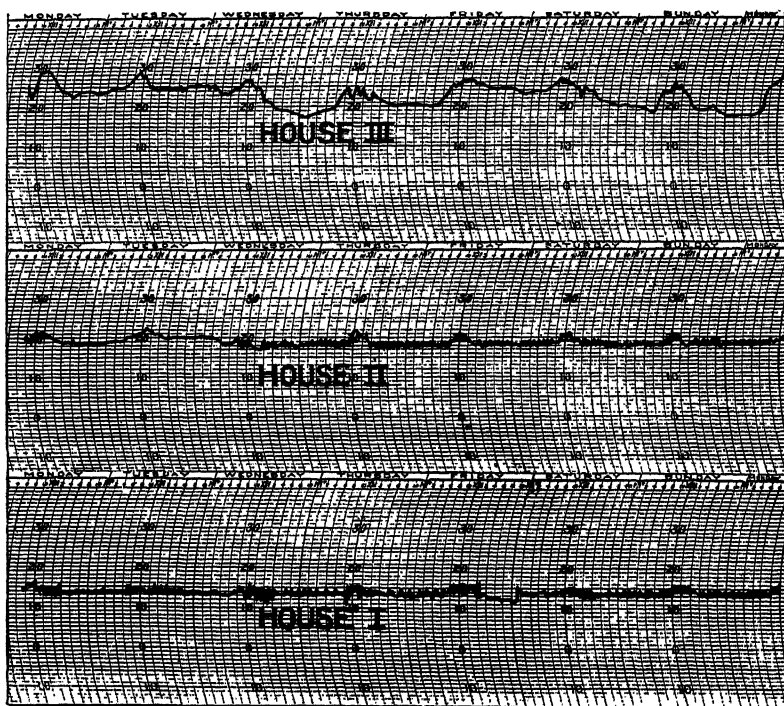


FIG. 4.—A weekly record of air temperatures kept in the course of the experiment showing the effect of different air temperatures on the development of cabbage yellows

With these results in mind, the following experiment was conducted with cabbage plants of Wisconsin Hollander and Commercial Hollander strains 77 days old. Lot 1 consisted of plants of both strains grown in healthy soil at air and soil temperatures of from 12° to 16° C., and lot 2 of plants grown at 25° to 28° air and soil temperature. Both lots were transferred to receptacles containing diseased soil as previously described.

The temperature of the air in the three greenhouse rooms was controlled to within fairly narrow limits except in the high-temperature house, where it could not be kept under perfect control during a season of exceptionally cold weather. Hourly temperature records were kept in three houses. Figure 4 shows a representative weekly

record for each of the three houses, and Figure 5 represents the mean daily temperature for each house during the 30 days of the experiment. It was planned to keep the high-temperature house at 28°, but the excessively cold weather brought the mean average down to 25°; the temperatures of the other houses were maintained at a mean average of 19° and 14.5°, respectively.

The results of this experiment are given in Table 5 and Figure 6. The disease developed more rapidly and in most cases was more severe in lot 1, plants grown at 12° to 16° C. before transplantation, than in lot 2, plants grown at 25° to 28° before transplantation. This is brought out strikingly in Figure 6, A, the plants in *b* (lot 2) being only slightly diseased while those in *a* (lot 1) were severely attacked, two of them having succumbed. This would seem to

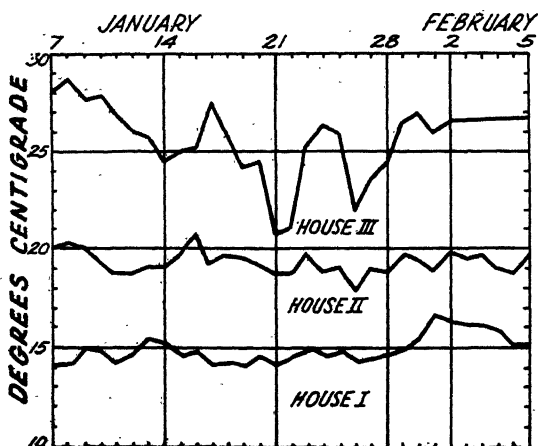


FIG. 5.—Mean daily air temperature in each of the three green-house rooms used during the experiment dealing with the effect of air temperature on the development of cabbage yellows

indicate that plants grown at higher temperatures before being transplanted to diseased soil are more resistant than those grown at lower temperatures.

Harvey (6), in his studies of the hardening process in plants, found that cabbage plants hardened for five days at 3° C. were rich in reducing sugars as glucose, and disaccharides as sucrose when compared with nonhardened plants grown at 18° to 25°, which contained a higher percentage of polysaccharides as starch. The hardened plants contained 7.64 per cent carbohydrates as compared with 5.16 per cent in the nonhardened plants. Lidforss (11) found this predominance of monosaccharides and disaccharides to be a common transformation in plants during the cold seasons. Hasselbring and Hawkins (7) found a similar condition in sweet potatoes kept at low temperatures. Rosa (12) found that the pentosan content of hardened cabbage plants is distinctly greater than that of nonhardened plants. Both of these investigators concluded that there is a slightly lower percentage of moisture in hardened cabbage plants than in plants grown at the higher temperatures, and Harvey (6) indicates that in the hardened plants there is an increase in total nitrogen and phosphorus.

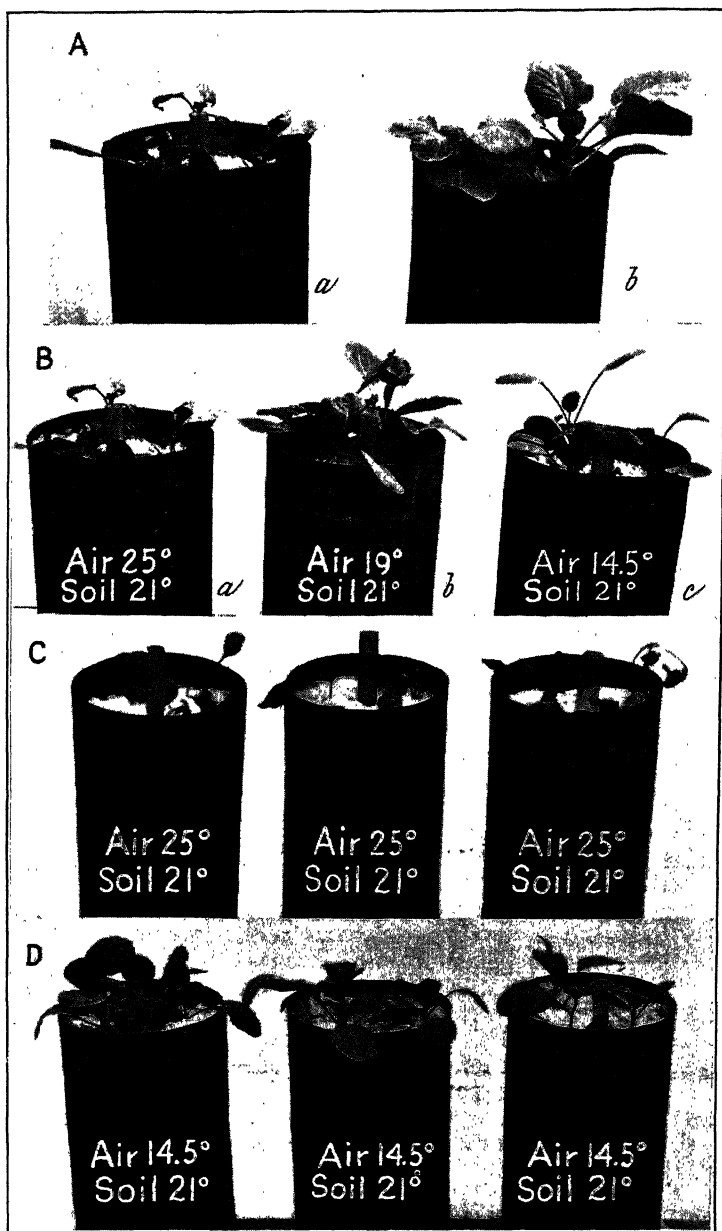


FIG. 6

A.—Commercial Hollander plants grown in healthy soil for 77 days before transplantation to naturally infested soil in tanks, at soil temperature of 21° C. and air temperature of 28°: *a*, Plants grown at 12° to 16° before transplanting; *b*, plants grown at 25° to 28° before transplanting. Note in *a* that all three plants are diseased and two are dead; in *b* two are very slightly diseased.

B.—Commercial Hollander plants kept in diseased soil for 30 days at a soil temperature of 21° C.: *a*, At air temperature of 25° all plants dead; *b*, at air temperature of 19° one plant diseased, the others healthy; *c*, at air temperature of 14.5° no disease evident.

C.—Wisconsin Hollander plants kept in diseased soil for 30 days at a soil temperature of 21° C. Of 9 plants kept at a daily average air temperature of 25°, 7 dead, 2 severely diseased.

D.—Wisconsin Hollander plants kept at a daily average air temperature of 14.5°, all plants healthy.

TABLE 5.—Effect of different soil and air temperatures upon the development of cabbage yellows

[Plants of lot 1 were grown at 12° to 16° C. for 55 days before transplantation to diseased soil; those of lot 2 were grown for the same length of time at 25° to 28°]

WISCONSIN HOLLANDER

Air temperature (° C.)	Soil temperature (° C.)	Lot 1					Lot 2				
		Total number of plants	Per cent diseased after 18 days	Per cent diseased after 24 days	Per cent diseased after 30 days	Per cent dead after 30 days	Total number of plants	Per cent diseased after 18 days	Per cent diseased after 24 days	Per cent diseased after 30 days	Per cent dead after 30 days
14.5	15	9	0	0	0	0	9	0	0	0	0
	21	9	0	22	22	0	8	0	0	0	0
	27	8	12	37	37	25	7	0	12	37	12
19	15	9	0	0	0	0	9	0	0	0	0
	21	8	12	24	60	37	0	0	0	0	0
	27	8	24	36	72	48	8	12	36	60	24
25	15	9	0	0	0	0	9	0	0	0	0
	21	8	24	48	74	36	8	0	12	24	12
	27	8	48	72	84	60	8	12	36	48	36

COMMERCIAL HOLLANDER

14.5	15	3	0	0	0	0	3	0	0	0	0
	21	3	0	0	0	0	3	0	0	0	0
	27	3	33	66	100	100	3	33	66	100	100
19	15	3	0	0	0	0	3	0	0	0	0
	21	3	0	33	66	66	3	0	0	33	0
	27	3	66	100	100	100	3	33	66	100	100
25	15	3	0	0	0	0	3	0	0	0	0
	21	3	100	100	100	100	3	0	33	66	66
	27	3	100	100	100	100	3	33	66	100	100

* Two plants in this lot showed only slight yellowing with no dwarfing, distortion, or shedding of leaves as is typical of the disease at the higher temperatures, but the fungus was found in the main stem and the petioles of the older leaves.

It is uncertain whether the changes taking place in cabbage plants exposed to relatively low temperatures bear any significant relation to the differences in percentages of disease among plants grown at higher temperatures. The temperature at which Harvey and Rose hardened their plants was much lower than that used in the experiment just described, 12° to 16° C. However, it seems likely that changes of the same general nature may occur in plants when grown at somewhat higher temperatures.

When the temperature of the soil was kept constant the rapidity with which yellows developed varied greatly with changes in the temperature of the air. At the highest soil and air temperature all of the Commercial Hollander plants of lot 1 were dead after 12 days, followed soon by lot 2 and by lot 1 of the Wisconsin Hollander plants, which were all diseased except 1 after 15 days. In the 19° house the incubation period of the disease lasted several days longer than in the 25° house, but was about the same as in the 14.5° house.

The effect of varying the air temperature while the soil temperature remains constant is well illustrated by Figure 6, B. Here the three receptacles were kept for 30 days at a uniform soil temperature of 21°. The plants in *a* were held at an average air temperature of 25°, those in *b* at 19°, and those in *c* at 14.5°. At the end of the 30 days all of the plants in *a* were dead; of those in *b* one was slightly diseased and the others were healthy; in *c* no disease appeared.

Figure 6, C and D, shows even more strikingly the influence of air temperature on yellows development. Both lots of the Wisconsin Hollander plants shown in this figure were kept at a soil temperature of 21°C ., but the plants in C were kept at an average air temperature of 25° , whereas those in D were kept at an average air temperature of 14.5° . In C all of the nine plants were dead or diseased, but in D the plants showed no symptoms of disease.

No disease symptoms were evident in the Commercial Hollander plants in either lot 1 or lot 2 in the 21° tank in the 14.5° house. But upon examination it was found that the vascular bundles of these plants were discolored and contained the mycelium of the yellows fungus up to 2 inches above the soil line. The following experiment was conducted to ascertain whether plants of the susceptible strains

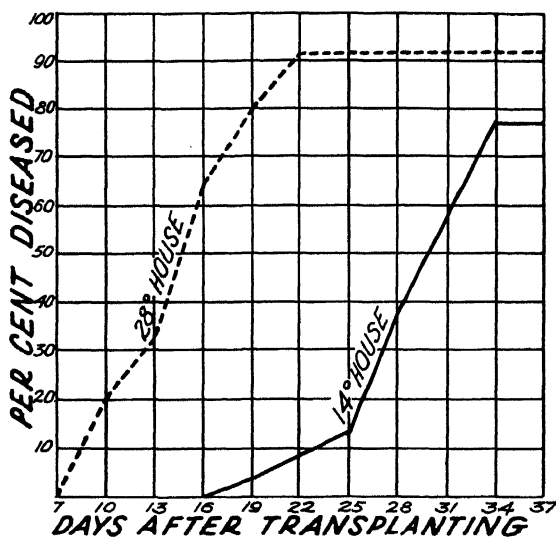


FIG. 7.—Comparison of the rate of development of yellows in two lots of Commercial Hollander cabbage seedlings kept at a constant soil temperature of 21°C . and air temperatures of 14° and 28° , respectively. (See Table 6)

would consistently exhibit this lack of typical yellows symptoms when grown at high soil temperature and low air temperature.

In each of 16 receptacles containing diseased soil were placed four plants of the Commercial Hollander strain 40 days old. Eight of these receptacles were then put in the 21° tanks of the 28° house and eight in the 21° tanks of the 14° house. In the 28° house yellows appeared on the eighth day, and by the twenty-second day 92 per cent of the plants were diseased; in the 14° house the first symptoms of disease appeared on the eighteenth day, after which the disease progressed slowly until the thirty-fourth day. The results of this experiment are shown in Table 6 and Figures 7 and 8. The difference in percentage of plants killed and the rate at which they were killed is pronounced. The death curve (fig. 8) parallels the disease curve (fig. 7) quite closely in the 28° house, but in the 14° house there is little similarity between the two. In this house the first plant was not killed until the twenty-sixth day, and after 37 days 72 per cent of the original number still survived.

TABLE 6.—Variations in susceptibility between two lots of Commercial Hollander cabbage seedlings grown at a soil temperature of 21° C. and air temperatures of 28° and 14° respectively

PER CENT DISEASED												
Air temperature (° C.)	Number of plants	After 7 days	After 16 days	After 13 days	After 16 days	After 19 days	After 22 days	After 25 days	After 28 days	After 31 days	After 34 days	After 37 days
28	32	0	20	32	64	80	92	92	92	92	92	92
14	32	0	0	0	0	4	8	12	36	56	76	76

PER CENT DEAD												
28	32	0	4	10	30	52	68	80	80	80	80	80
14	32	0	0	0	0	0	0	0	4	4	16	28

In all of the experiments conducted by the writer no case was found where the yellows disease developed at a soil temperature as low as 15° C. Since the yellows fungus is found in the roots of many plants grown at a soil temperature of 15°, it was believed that if the

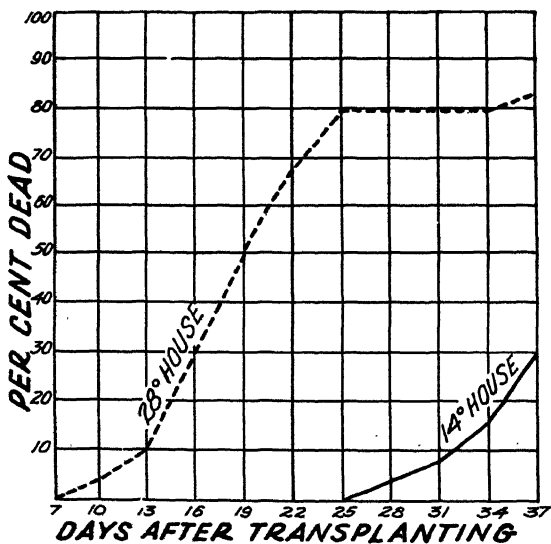


FIG. 8.—Comparison of the death rate in two lots of Commercial Hollander cabbage seedlings kept at a constant soil temperature of 21° C. and air temperature of 14° and 28°, respectively. (See Table 6)

plants were grown at a high air and low soil temperature the fungus might penetrate the parts of the plants exposed to the warm air, but experiment proved that such was not the case. The fungus was found to be present in the tap roots of many of the plants tested but never above the soil line in the stems.

In the experiment next to be described the soil moisture was kept at about 27 per cent of the dry weight, or 60 per cent of the moisture-holding capacity. Wisconsin Hollander and Commercial Hollander plants grown in healthy soil for 62 days at 20° to 23° C. were trans-

planted to receptacles containing naturally infested soil, two plants to each can. These plants were healthy in appearance and about the size of those commonly used for transplanting to the field. The plants were kept for 30 days at soil temperatures ranging from 15° to 33° in increments of 3°.

TABLE 7.—*Comparative resistance to Fusarium conglutinans of Wisconsin Hollander and Commercial Hollander cabbage plants grown in naturally infested soil at 60 per cent moisture-holding capacity for 30 days*

WISCONSIN HOLLANDER													
Soil temperature (°C.)	Number of plants	Per cent diseased											
		After 12 days		After 15 days		After 21 days		After 24 days		After 27 days		After 30 days	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
15	10	0	0	0	0	0	0	0	0	0	0	0	0
18	10	0	0	0	0	0	0	0	0	0	0	0	0
21	10	0	0	0	0	0	0	0	0	0	0	0	0
24	10	0	0	0	0	0	0	0	0	0	0	10	0
27	10	0	0	0	0	0	0	0	0	0	0	10	0
30	10	0	0	20	0	30	0	20	0	40	0	40	0
33	10	0	0	0	0	0	0	0	0	0	0	10	0

COMMERCIAL HOLLANDER													
15	6	0	0	0	0	0	0	0	0	0	0	0	0
18	6	0	0	0	0	0	0	0	0	0	0	0	0
21	6	0	0	0	0	0	0	0	0	33	0	50	0
24	6	17	0	33	0	83	17	83	33	100	50	100	83
27	6	0	0	33	0	50	0	50	33	100	50	100	100
30	6	33	0	100	0	100	66	100	100	100	100	100	100
33	6	17	0	17	0	17	0	33	0	33	17	67	33

The results of this experiment, given in Table 7 and Figure 9, are strikingly different from those in most of the preceding work. Here for the first time are conditions in which the differences in resistance between the plants of the so-called "resistant" and "susceptible" strains approximate those in the field. A much smaller percentage of disease was present in the plants of the resistant strain at all temperatures favorable for its development, and the number of deaths was strikingly fewer. Although at 27° and 30° there was a comparatively high percentage of disease in the Wisconsin Hollander plants, the amount of disease fell off rapidly at 24° and 33°, and at 21° none of the 10 plants was diseased. But at 24°, 27°, and 30° all of the susceptible plants were diseased, the number of diseased plants decreasing rapidly below 24°. At 18° there was no disease in either strain.

Something of this difference in susceptibility between the resistant and commercial strains is brought out in Figure 10. The three receptacles on the left in each case contain Commercial Hollander plants and the three on the right contain Wisconsin Hollander. Of the resistant plants shown in Figure 10, A (right), one was slightly diseased, whereas of the susceptible plants four were diseased and one was dead. At 30° (B) the disease was fatal to all plants of the susceptible strain and to two of the resistant strain, the other four

being diseased. At temperatures of 27°, 24°, and 21° the difference in susceptibility is so obvious as to need no discussion.

These results may be correlated with those obtained under field conditions. In general, the mean average soil temperature during the critical period of growth of the cabbage, June, July, and August, at Racine, Wis., lies between 18° and 24° C. If the mean average soil temperature remains about 20° to 21° there is little disease in the plants of resistant strains and from 40 to 80 per cent in those of the

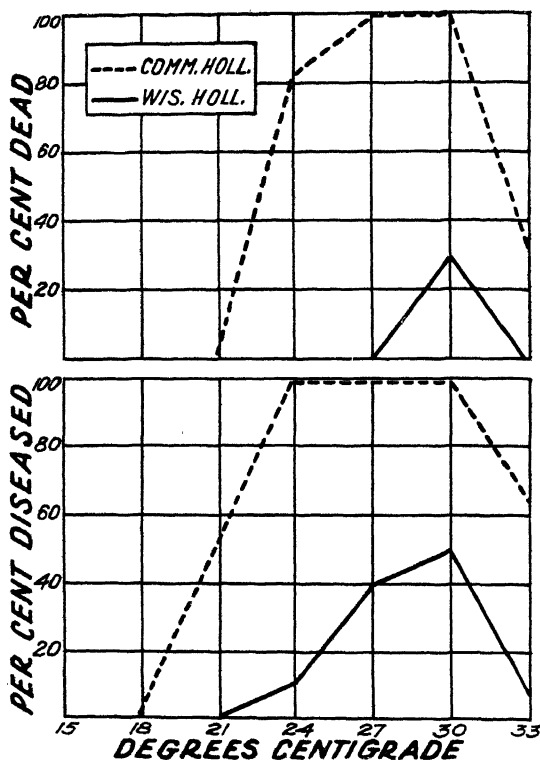


FIG. 9.—Comparison of yellows development in Wisconsin Hollander and Commercial Hollander plants grown at different temperatures for 30 days in naturally infested soil

susceptible strains. If the temperature goes up to about 24° the percentage of disease goes up also, these changes depending somewhat upon moisture as well as upon soil-temperature conditions.

RELATION OF SOIL MOISTURE TO THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS

Only one experiment was conducted to test the effect of soil moisture on the development of yellows in cabbage, and the results obtained were too variable to be of much value. But it may be mentioned that these results are in accord with those of Tisdale (14), who found that the disease is more severe in soils of comparatively low moisture content. Under conditions of proper soil moisture the cabbage plants seem to make better growth and are more resistant to the attack of the yellows fungus.

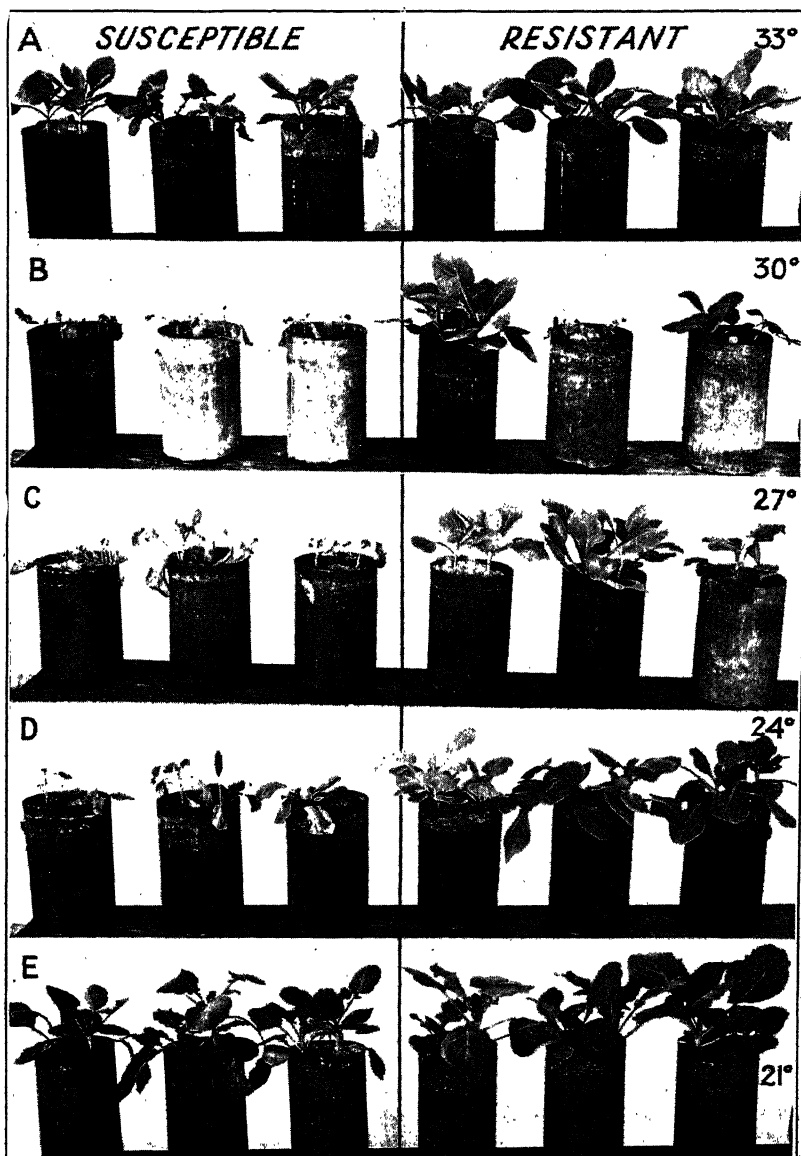


FIG. 10.—Wisconsin Hollander and Commercial Hollander plants grown in healthy soil for 62 days before being transplanted to diseased soil kept at 60 per cent of the moisture-holding capacity for 30 days at different temperatures. The three receptacles on the right contain in each case six plants of the resistant strain and the three on the left contain in each case six of the susceptible strain

A.—Soil temperature, 33° C. One of the resistant strain and four of the susceptible strain diseased; one of the latter strain dead

B.—Soil temperature 30° C. Four of the resistant variety diseased, two dead; all of the susceptible variety dead

C.—Soil temperature, 27° C. Four of the resistant strain slightly diseased, all the susceptible plants dead

D.—Soil temperature, 24° C. One of the resistant strain diseased, all of the susceptible strain dead

E.—Soil temperature, 21° C. All of the resistant strain healthy, and three of the susceptible variety diseased

It was noted by Jones and Gilman (8) and later by Tisdale (14) that severe attacks of yellows are associated with hot, dry weather. Gilman (5) showed that the incubation period of the disease ranged from 14 days, when the mean daily temperature 6 inches below the soil was 23°C ., to 20 days when the mean daily temperature at the same depth was about 20° . In 1919 Tisdale transplanted Commercial Hollander seedlings to a soil where the daily temperature 4 inches below the surface was 25° , and 13 days later he found 30 per cent diseased. He concluded that the length of the incubation period might be as low as 12 days when the mean soil temperature was 20° .

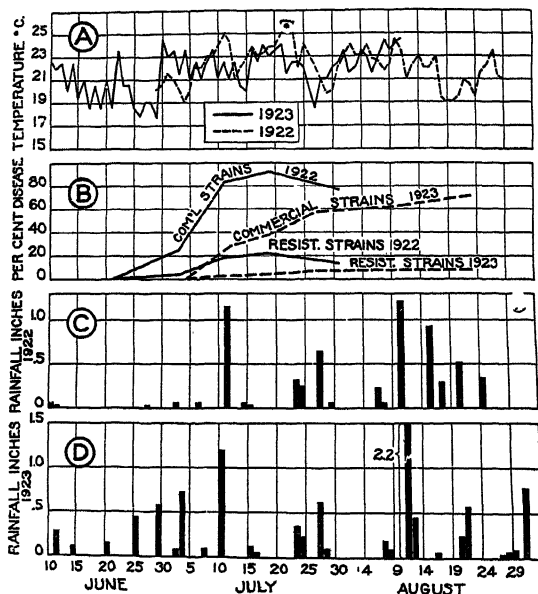


FIG. 11.—Summary of data from experimental plots at Racine, Wis., for June, July, and August, 1922 and 1923. A, mean average daily soil temperature at a depth of 4 inches; B, percentage of diseased plants in both resistant and susceptible strains; C and D, daily precipitation

He also found that lowering the moisture content of the soil increased to a considerable extent the quantity of disease.

Observations on the development of yellows in the experimental plots at Racine, Wis., in 1922 and 1923 made by the writer tend to corroborate those of Gilman and Tisdale. It was noted in 1922 that during a hot, dry period about the middle of July a comparatively high percentage of yellows developed in some of the most resistant strains, but that during the latter part of the month when the soil temperatures were considerably lower and rain fell abundantly many of the diseased plants completely recovered, and a little later when the counts of disease were made the actual percentage of yellows was lower than in the same plots two weeks earlier.

In correlating the temperature of the soil with the development of yellows in the experimental plots in 1922 and 1923, an attempt was made to ascertain whether the quantity of rainfall during July and August, the critical period for the development of the disease, might not have influenced the occurrence of *Fusarium* in the seedlings.

The results were not conclusive, but they rather indicate that the total rainfall during these months is a factor in the development of yellows. In 1922 the mean soil temperature for June, July, and August was about the same as for the same period in 1923, but the quantity of rainfall was much smaller in 1922, and the percentage of yellows in the experimental plots was much greater.

In 1922 some 32 strains were tested and in 1923 the number was increased somewhat, but only the more representative are included in the data given in Tables 8 and 9. The average percentage of disease in the resistant and susceptible strains is shown in Figure 11, B; the mean average soil temperatures at a depth of 4 inches from the surface for June, July, and August, 1922 and 1923, are given in Figure 11, A; and the total daily precipitation in inches, as taken from the United States Weather Bureau records (Racine office) for the same months in 1922 and 1923 (15), are shown in Figure 11, C and D.

Three features of Figure 11 are apparent at a glance: (1) The mean average soil temperature for the critical periods of 1922 and 1923 are very similar, the mean for the period being 21.1° C. in 1922 and 21.3° in 1923; (2) the amount of rainfall during the summer of 1922 was considerably less than that of 1923; and (3) the average percentage of yellows in both the resistant and susceptible strains was higher in 1922 than in 1923. Especially is this difference in disease evident in the resistant strains, the actual percentage being 13 in 1922 and 7.4 in 1923. This would seem to indicate that the decreased rainfall during the summer of 1922 might account in part at least for the increased percentage of disease over that of the following year.

TABLE 8.—*Summary of field observations on the experimental plots at Racine, Wis., 1922*

Strains	Total number of plants	July 3		July 11		July 19		July 31	
		Number diseased	Per cent diseased	Number diseased	Per cent diseased	Number diseased	Per cent diseased	Number diseased	Per cent diseased
Susceptible:									
All Seasons.....	254	31	12.0	153	60.0	188	74.0	180	70.0
Glory.....	246	47	19.0	181	73.0	217	88.0	210	85.0
All Head No. 1.....	247	66	26.0	196	79.0	197	79.0	186	75.0
All Head No. 2.....	89	38	42.0	78	87.0	82	92.0	74	83.0
Copenhagen No. 1.....	252	78	31.0	237	94.0	248	98.0	227	90.0
Copenhagen No. 2.....	119	66	55.0	114	96.0	118	99.0	117	98.0
Copenhagen No. 3.....	247	65	26.0	239	96.0	240	97.0	221	89.0
Copenhagen Iowa No. 5.....	121	10	8.0	52	43.0	55	45.0	60	50.0
Resistant:									
W. H. 9-21 (1-3).....	555	3	.6	12	2.1	21	3.8	4	.7
W. H. 8-21 (A-B).....	248	1	.4	10	4.0	13	5.2	8	3.2
XXV-20-A.....	483	1	.2	26	5.4	35	7.2	19	3.9
XXV-20-B.....	369	9	2.4	95	25.7	150	40.6	85	23.0
XXV-21-B.....	375	1	.3	40	10.6	68	18.1	30	8.0
XI-20 (1-2).....	498	3	.6	76	15.2	124	24.9	56	11.2
XI-21 (2-5).....	1,427	124	8.7	382	26.7	419	29.3	247	17.3
XXXV-20-4.....	349	44	12.6	130	37.2	145	41.5	107	30.6

TABLE 9.—Summary of field observations on the experimental plots at Racine, Wis., 1923

Strains	Total number of plants	July 11		July 18		July 26		Aug. 9		Aug 22	
		Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow
Susceptible:											
Glory.....	652	188	29.0	294	45.0	440	67.0	462	71.0	466	71.0
All Head.....	351	116	33.0	141	40.0	205	58.0	234	66.0	292	83.0
Mammoth Rock											
Red.....	366	63	17.0	104	28.0	113	30.0	140	38.0	190	52.0
Hollander.....	254	48	19.0	95	37.0	211	83.0	230	90.0	238	93.0
Brunswick.....	31	10	32.0			21	68.0	28	90.0	28	90.0
Large Blood Red	26	4	15.0	4	15.0	8	31.0	12	46.0	18	69.0
Resistant:											
W. H. 9-22-1.....	360	0	.0	3	.8	11	3.0	17	4.7	23	6.4
W. H. 8-22-A.....	170	0	.0	0	.0	1	.5	5	2.9	5	2.9
W. H. 8-22-D.....	253	1	.4	1	.4	3	1.2	17	6.7	28	11.0
XXV-22-A.....	645	2	.4	2	.4	2	.4	2	.4	2	.3
XXV-22 (1-6).....	3,095	14	.5	18	.6	60	1.9	74	2.4	83	2.6
XXIV-22-2, 3, 4, 8.....	217	1	.4	1	.5	0	.0	0	.0	0	.0
XL-22-1.....	1,441	0	.0	0	.0	0	.0	0	.0	0	.0
XL-23-G. C.....	113							20	17.7	41	36.3
Red Nos. 1 and 2.....	809	13	1.6	20	2.5	58	7.2	60	7.4	63	7.8
XXXV-22-1.....	983	60	6.1	81	8.2	111	11.2	132	13.4	148	15.1
XXXV-22-2.....	1,179	64	5.4	81	6.9	158	13.4	165	14.0	178	15.1
XXXV-22-6.....	1,432	65	4.5	100	7.0	165	11.5	196	13.7	220	15.4
XXX-22-1.....	976	49	5.0	75	7.7	113	11.6	123	12.6	144	14.8
XXX-22-2.....	798	38	4.7	50	6.3	79	9.9	100	12.5	111	13.9
XXX-22-4.....	710	30	4.2	41	5.7	52	7.3	61	8.6	67	9.4

SUMMARY

In the experiments herein described the writer has studied the effect of environmental factors upon the expression of the resistant quality in cabbage exposed to attacks by the fungus *Fusarium conglutinans*.

It was found that young seedlings of Wisconsin All Seasons and Commercial All Seasons strains were almost equally susceptible to yellows up to the age of 20 days.

A high percentage of plants of the most resistant varieties became diseased when placed under conditions of low soil moisture and relatively high soil temperature, 27° to 33° C., but from 21° to 24°—the temperatures nearest those encountered in the field in the Racine, Wis., cabbage-growing section—the “resistant” varieties contracted the disease only slightly. No evidence of yellows development was noted at a soil temperature of 15° and only a comparatively small percentage developed at 18°.

Cabbage plants of both “resistant” and “susceptible” strains grown in healthy soil at 12° to 16° C. before transplantation to diseased soil at a higher temperature (27°) proved to be more susceptible to yellows than plants grown at 25° to 28°.

Air temperature affects yellows development independently of soil temperature. Wisconsin Hollander and Commercial Hollander plants were grown in naturally infested soil at soil temperatures of 15°, 21°, and 27° C. in each of three greenhouse rooms kept at 14.5°, 19° and 25°, respectively, for 30 days. At a soil temperature of 27° the disease developed most rapidly in the 25° chamber and slightly more slowly in the 19° and 14.5° chambers, but the final percentage

of disease was about equal in the three. At a soil temperature of 21° the disease developed most rapidly in the 25° room and less rapidly in the 19° room, and the disease was very slight in the 14.5° room.

Commercial Hollander plants were grown in diseased soil at a soil temperature of 21° C. and at air temperatures of 14° and 28°. Yellows developed much more rapidly, a larger percentage of plants were diseased, and a much greater number of plants were killed by the disease at an air temperature of 28° than 14°.

A study of field conditions for two years and a limited amount of greenhouse experimentation show that the quantity of rainfall apparently affects the amount of disease independently of soil temperature relations. In the summer of 1922 the soil temperatures at Racine, Wis., averaged somewhat lower than in 1923, but the total precipitation was considerably less in 1922 and the average percentage of disease was considerably higher.

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PHYSIOLOGICAL STUDIES OF THE EFFECT OF ARSENICALS ON THE RESPIRATORY METABOLISM OF INSECTS¹

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INTRODUCTION

In previous investigations (7)³ it was established that during the embryonic development and metamorphosis of insects, the average oxygen consumption and carbon dioxide output, when plotted against time, produced graphs which in general indicated an increase in the gaseous exchange from a minimum during early development to a maximum as developmental processes terminated. The substance chiefly utilized as energy for an upbuilding of the organism in the metabolic process was found to be reserve fat. It was also shown that in consequence of histolysis the average results of the respiratory gas exchange when plotted as above produced graphs of a reverse order, indicating a maximum gaseous exchange at the beginning of histolysis and a minimum exchange as the histolytic process terminated. Recently the type of metabolic activity that functions during starvation and hibernation of insects has also been indicated (8). It appeared, therefore, of considerable interest in a study of arsenicals to observe their action upon respiratory metabolism.

If the information from such a study substantiates a relationship between susceptibility and physiological or metabolic activity, it may be possible to distinguish a degree of susceptibility to arsenical poisoning of tissues and organs, and to elucidate the factors involved in acquired tolerance of organisms to low concentrations of poison.

In the literature, references to the influence of arsenicals upon the respiratory metabolism of insects appear to be entirely lacking. Shafer (11, 12) has indicated the inhibiting influences which substances like gasoline, kerosene, carbon disulphide, hydrocyanic-acid gas, sodium fluoride, and others exert upon respiration and upon oxidases, catalases, and reductases. He found that the respiratory activity of treated insects increased until they became deeply affected, after which it was depressed. These gases also caused the value of the respiratory quotient to rise above that of normal animals. The conclusion derived was that these vapors depress the O₂ absorption more than they do the CO₂ excretion. Child (4), Hyman (9), and others have verified the inhibiting effect of potassium cyanide (KCN) upon the gaseous exchange in *Planaria*. Cunze (6) found a

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³ Reference is made by number (*italic*) to "Literature cited," p. 1007.

reduction of the gaseous exchange as a result of the injection of arsenic trioxide in rabbits.

In the present investigations an attempt has been made to determine the influence that arsenical compounds and their component oxides and arsenic contents have upon the respiratory metabolism of insects.

MATERIAL AND METHODS

The material employed in these investigations consisted of the adult potato beetle, *Leptinotarsa decemlineata* Say, the garden wireworm, *Pheletes agonus* Say, and third-stage larvae of the Japanese beetle, *Popillia japonica* Newman.

Measurements of respiratory metabolism were made with a Krogh's manometer as modified by Bodine and Orr (1). Further minor modifications made by the writer consisted in widening the diameter of the shank to accommodate wider-mouthed respiratory chambers, in fusing a glass hook to the bottom of the shank from which the insect was suspended, and in increasing the length of the capillary tubes. This last modification enabled determinations to be made with comparatively large insects.

It is essential in respiratory investigations that basal metabolism be determined; hence the voluntary movements of insects under experimentation were controlled by confining the insects in cotton gauze and tying them to eliminate body movements or muscle tonus. Wireworms, however, persistently worked themselves loose from gauze, and it was found convenient to use instead pieces of heavy unbleached muslin, which did not interfere with their normal respiration. The insects with body movements thus controlled were suspended from the small glass hook in the respiratory chamber of the manometer. They could be left in the chamber for 2 to 10 hours as the nature of the experiment demanded. Constant temperatures were maintained by the use of a water bath in which the respiratory chambers of the manometer were immersed.

The procedure in all investigations was first to determine the normal rates of metabolism of an insect and afterwards to feed it poisoned foliage, or to place a known quantity of an arsenical solution on the mouth parts to be absorbed by the insect, or to inject in the oral cavity the quantity of solution to be tested. In some experiments the insects were left on poisoned foliage for only a few hours and in others they were left from 10 to 16 hours before their metabolic rates were again determined.

Uniform suspensoid solutions of lead arsenate, calcium arsenate, manganese arsenate, and ferric arsenate were made by using 0.145 gm. of the arsenical per 100 cubic centimeters of water (5). The calculations were based upon the average arsenic oxide content (32 per cent) present in the arsenical, such proportions being the equivalent of those used in field control of insects, namely, 2 pounds of the arsenical to 50 gallons of water. The prepared solutions were sprayed on potato foliage by means of a fine syringe and the foliage was then fed to insects. For the purpose of uniformity only those insects were selected for metabolism determinations that indicated a consumption of poisoned foliage equivalent (approximately) to that consumed by insects that were feeding on foliage sprayed with a different arsenical. The results obtained from such experiments gave the effect produced by the arsenical compound on respiratory metabolism.

In determining the effect of the oxide component of the arsenical compound upon respiration, neutral suspensoid solutions (0.001 gm. per cubic centimeter of water) of lead oxide, calcium oxide, manganese oxide, and ferric oxide were prepared and fed to insects by means of a small graduated pipette having the tip drawn out into a fine capillary,⁴ similar in principle to the micro burette described by Campbell (2). A definite quantity of liquid (0.01 c. c.) was used in each case and was placed on the mouth parts of the insect in minute droplets, the liquid flowing from the pipette by gravity or slight mouth pressure at a sufficiently slow rate to be absorbed by the insect.

For the purpose of determining the effect of the arsenical alone upon respiratory metabolism, neutral solutions of arsenious acid and of arsenic acid were fed to the insects by means of the graduated pipette already mentioned and also by means of a small hypodermic syringe, a definite quantity being injected in the oral cavity of the insect.

Realgar (As_2O_3) and manganese arsenate were utilized chiefly because of their color property. It was thought that as portions of the poison upon which the insects fed became absorbed and distributed in the body, their color (realgar is red, manganese arsenate is grayish brown) might prove of some aid in distinguishing their location in tissues and organs, but subsequent histological investigations of insects fed on these agents did not substantiate this view.

EXPERIMENTAL DATA

For the sake of brevity and clearness the data of the experiments have been averaged and summarized in the accompanying tables and figures. For the methods used in calculating the results the reader is referred to the article by Krogh (10).

TABLE 1.—Average respiratory gas exchange of female potato beetles to which arsenicals were fed for 16 hours

Group No. ^a	Kind of feeding	O ₂ intake	Probable error \pm	CO ₂ output	Probable error \pm	CO ₂ / O ₂	Probable error \pm	Per cent depression ^b	
								O ₂	CO ₂
		C. c.		C. c.					
L ₁	Normal.....	64.00	1.85	37.15	5.03	0.580	0.029		
	Lead arsenate.....	58.59	7.01	42.60	5.74	.727	.025	8	+12
L ₂	Normal.....	97.48	4.92	61.29	7.20	.628	.013		
	Lead arsenate.....	30.91	8.34	15.62	2.75	.505	.029	68	74
L ₃	Normal.....	46.40	2.64	26.10	3.45	.562	.024		
	Lead arsenate.....	50.50	3.62	37.00	2.79	.732	.019	+8	+29
L ₄	Normal.....	56.80	2.14	44.70	2.57	.787	.015		
	Lead arsenate.....	55.70	5.40	41.40	2.29	.743	.037	2	7
L ₅	Starving.....	31.70	1.90	15.60	2.12	.492	.017		
	Lead arsenate.....	24.10	2.70	10.70	3.74	.444	.033	24	31
L ₆	Normal.....	110.68	8.60	87.15	5.66	.787	.013		
	Manganese arsenate.....	68.23	9.36	40.80	7.40	.597	.033	38	54
L ₇	Normal.....	113.42	2.15	83.28	2.58	.734	.024		
	Realgar.....	124.90	2.96	79.65	2.20	.637	.017	+9	4
L ₈	Normal.....	105.66	2.50	83.49	15.00	.790	.027		
	Calcium arsenate.....	66.30	6.70	47.80	6.80	.721	.025	37	42
L ₉	Normal.....	136.45	4.50	99.20	3.80	.727	.019		
	Ferric arsenate.....	114.15	3.20	81.70	2.70	.717	.021	16	17

^a The pipette used by the writer was made of glass tubing 10 cm. long and 2 mm. inside diameter, the tip drawn into a fine capillary. A strip of millimeter paper was pasted the length of the tube and the pipette was calibrated with mercury. The liquid flowed by gravity, or by mouth pressure when a compensating tube was connected by rubber tubing to the capillary.

^b L₁, L₂, L₄, old beetles; L₃, L₆ to L₉, young beetles; L₅, starving beetles.

^c By "depression" is meant the deviation from normal metabolism attributable to the poison.

^d Plus sign before figure indicates an increase in percentage.

TABLE 2.—Average respiratory gas exchange of male potato beetles to which arsenicals were fed for 16 hours

Group No. ^a	Kind of feeding	O ₂ intake	Probable error \pm	CO ₂ output	Probable error \pm	CO ₂ O ₂	Probable error \pm	Per cent depression ^b	
								O ₂	CO ₂
		<i>C. c.</i>		<i>C. c.</i>					
L ₁	Normal.....	65.52	3.11	41.99	7.20	0.641	0.043		
	Lead arsenate.....	63.16	1.19	44.80	5.10	.709	.046	3	^c +6
L ₂	Normal.....	116.80	1.35	78.09	1.56	.668	.023		
	Lead arsenate.....	33.94	1.89	31.14	3.54	.917	.019	71	60
L ₃	Normal.....	66.00	2.76	47.70	2.16	.723	.021		
	Lead arsenate.....	56.80	1.54	38.90	3.42	.685	.025	13	18
L ₄	Starving.....	61.55	2.86	40.90	3.01	.664	.026		
	Lead arsenate.....	37.02	3.21	22.10	1.92	.596	.038	40	46
L ₅	Normal.....	107.60	2.55	77.05	4.20	.716	.034		
	Manganese arsenate.....	61.00	3.20	41.20	2.15	.675	.061	43	46
L ₆	Normal.....	148.50	2.67	115.00	3.50	.774	.024		
	Realgar.....	160.10	2.21	132.00	2.30	.824	.016	+7	+12
L ₇	Normal.....	71.15	3.45	69.13	2.43	.971	.025		
	Calcium arsenate.....	18.10	3.20	18.10	1.76	1.000	.011	74	73
L ₈	Normal.....	92.60	2.10	74.10	1.87	.800	.017		
	Ferric arsenate.....	50.80	3.40	33.60	2.37	.661	.019	45	55

^a L₁, L₂, old beetles; L₂, L₃ to L₈, young beetles; L₁, starving beetles.^b By "depression" is meant the deviation from normal metabolism attributable to the poison.^c Plus sign before figure indicates an increase in percentage.

TABLE 3.—Percentage deviation from normal of the gas exchange of first-generation potato beetles fed different arsenicals

Poison used	Sex of beetles	O ₂ intake	CO ₂ output	CO ₂ O ₂
Lead arsenate.....	Female.....	-68	-74	-19
Do.....	Male.....	-71	-60	+18
Calcium arsenate.....	Female.....	-37	-42	-8
Do.....	Male.....	-74	-73	+3
Manganese arsenate.....	Female.....	-38	-54	-24
Do.....	Male.....	-43	-46	-5
Ferric arsenate.....	Female.....	-16	-17	-1
Do.....	Male.....	-45	-55	-17
Realgar.....	Female.....	+9	+4	-13
Do.....	Male.....	+7	+12	+6

Tables 1 to 3 indicate the average oxygen consumption, carbon dioxide production, and respiratory quotient⁵ resulting from normal feeding, and the rates after a certain period of feeding on sprayed foliage. The deviation from normal metabolism attributable directly to poisoned food has been calculated in terms of percentage and placed in a separate column ("Per cent depression"). These figures give a clear conception of the effect of the poison upon the insects under experimentation.

RESULTS WITH LEAD ARSENATE

Experimentally, the oxygen consumption and the carbon dioxide output of first-generation beetles (young insects) after feeding on poisoned foliage were persistently and profoundly lowered (Tables 1 to 3). The reduction varied with different individuals; the average decline for young females (Table 1) was 68 and 74 per cent, and for

⁵ By "respiratory quotient" is meant the ratio of the CO₂ output to the O₂ intake, and is usually designated as $\frac{\text{CO}_2}{\text{O}_2}$.

young males (Table 2) 71 and 60 per cent. Old beetles after feeding on sprayed foliage gave different results. In females the average oxygen intake of three groups (L_1 , L_3 , and L_4) as compared to normal rates was practically unaffected; the CO_2 output, on the other hand, rose 17 per cent. In males the oxygen consumption decreased 8 per cent and the CO_2 output decreased 12 per cent. A marked elevation in the respiratory quotient of young males (27 per cent) was noted in these tests, whereas in young females a decline of 19 per cent occurred. No significant rise in the quotient of old males took place (4 per cent); old females, however, exhibited an increase of 19 per cent (fig. 1).

The effect that starvation may have on susceptibility to arsenical poisoning was also investigated. Male beetles, starved for a week

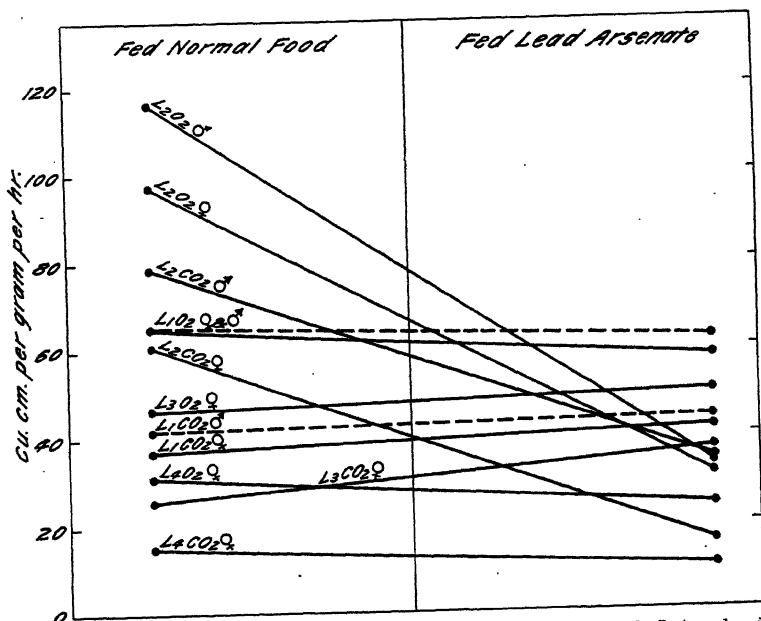


FIG. 1.—Normal rates of respiratory metabolism of four groups of potato beetles and effect produced on metabolism by the consumption of lead arsenate. L_1 and L_2 , old beetles; L_3 , young beetles; L_4 , starving beetles. Note the intense inhibiting effect of the poison on young as compared to old beetles

and afterwards placed on sprayed foliage, registered an intense lowering of the oxygen intake and CO_2 output, amounting to 40 and 46 per cent, respectively. In females the reduction amounted to 24 and 31 per cent. The theory that starvation may lead to physiological youthness would seem to be upheld by the susceptibility of starved beetles to arsenical poisoning, a susceptibility comparable to that of young beetles, as discussed above.

When potato beetles survived for three days the effects of feeding on sprayed foliage, it was possible to note the influence which such feeding produced on metabolism. From Figure 6, D, it will be observed that a gradual lowering of the oxygen intake occurred during the entire period of the experiment, whereas a diminution of the CO_2 output took place during the first day, followed by a slight rise on

the second day, a more decided reduction on the third day, and finally by the death of the individual. In the same figure the curve for the respiratory quotient is gradually elevated during the first two days, indicating that the substances catabolized were entirely different from those in normal metabolism. From the high quotient (0.8) and the dissolution of muscle tissues noted frequently in dissecting poisoned insects before death, it is surmised that muscle tissue destruction or protein metabolism very largely took place.

The average loss in weight sustained by 37 individuals which fed on foliage sprayed with lead arsenate amounted to 13 per cent of their total weight. Contrary to expectation, this reduction was not so great as that sustained by beetles which were fed foliage sprayed with other arsenicals, as will be discussed later. In other experiments grains of corn soaked in arsenic acid solutions (0.0115 gm. per cubic centimeter) were placed with wireworms, and after the wireworms had fed on the poisoned grain, measurements were made of the respiratory exchange. The oxygen intake and the CO₂ output were found to be reduced 24 and 18 per cent, respectively (Table 4). An increase of 6 per cent in the quotient was registered, but this is not considered significant.

TABLE 4.—Average respiratory gas exchange of wireworms which were fed arsenicals

Kind of feeding	O ₂ in- take	Prob- able error±	CO ₂ out- put	Prob- able error±	CO ₂ O ₂	Prob- able error±	Per cent depression	
							O ₂	CO ₂
Normal.....	C. c. 121.20	4.10	C. c. 86.50	3.20	0.714	0.092		
Lead arsenate.....	91.70	2.90	70.10	4.80	.764	.084	24	18
Normal.....	91.30	3.10	77.50	2.80	.848	.047		
Calcium arsenate.....	76.10	3.80	71.20	1.90	.936	.036	16	8

RESULTS WITH CALCIUM ARSENATE

First-generation females after feeding on foliage sprayed with calcium arsenate registered a lowering in oxygen intake and CO₂ output of 37 and 42 per cent, respectively (Table 1); first-generation males showed a reduction of 74 per cent oxygen intake and 73 per cent CO₂ output (Table 2). Figure 2 gives a graphic representation of the results based on an average of 21 individuals. The influence of calcium arsenate on metabolism when continued for a period of two days is shown in Figure 6, A. It should be explained that the gradual elevation of the oxygen intake as shown in this figure is attributable to the age of the insects and is comparable to the curve shown in Figure 1 for old beetles. The increase in CO₂ output occurs only on the first day and is followed by a marked depression on the second day. The quotient, however, continues to ascend for the entire period of the experiment, presumably indicating that the substances catabolized are similar in nature to those discussed under lead arsenate and are entirely different from those in normal metabolism. The average loss in weight sustained by 18 insects amounted to 16 per cent of their total weight.

Tests similar to those with lead arsenate were conducted with calcium arsenate on wireworms (Table 4). A decrease in the oxygen

consumption and CO_2 output of 16 and 8 per cent, respectively, is here shown. A significant rise in the quotient (9 per cent) took place.

RESULTS WITH MANGANESE ARSENATE

The feeding of foliage sprayed with manganese arsenate likewise profoundly decreased the oxygen intake and CO_2 output of both sexes, as shown in Figure 3. The depression of the gaseous exchange in females averaged 38 and 54 per cent, respectively (Table 1), in males 43 and 46 per cent (Table 2), and a decline in the quotient amounted in females to 24 per cent and in males to 5 per cent.

Figure 6, B, shows the results of experiments in which manganese arsenate affected potato beetles for a period of four days. It will

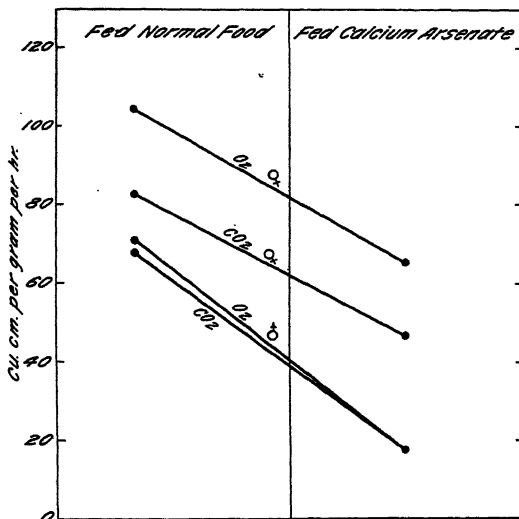


FIG. 2.—Normal rates of respiratory metabolism of first-generation male and female potato beetles, and effect produced on metabolism by the consumption of calcium arsenate

be noted from the curves in this figure that a depression of the gaseous exchange occurred during the first two days, followed by an increase on the third day. A considerable decline in the CO_2 output took place just before the death of the insects. The curve for the respiratory quotient parallels for most of its length the curve indicated for CO_2 production. The average loss in weight sustained by 28 beetles, attributable to arsenical feeding, amounted to 26 per cent. The curves in Figure 6, F, which are comparable in many respects to those for the adults (fig. 6, B), represent the reactions of larvae to similar tests.

RESULTS WITH FERRIC ARSENATE

With ferric arsenate the oxygen consumption and CO_2 production of females decreased only 16 and 17 per cent, respectively, but with males the depression was 45 and 55 per cent (Tables 1, 2, and 3 and fig. 4). The respiratory quotient of females remained practically normal, whereas in males it decreased 17 per cent. It is very

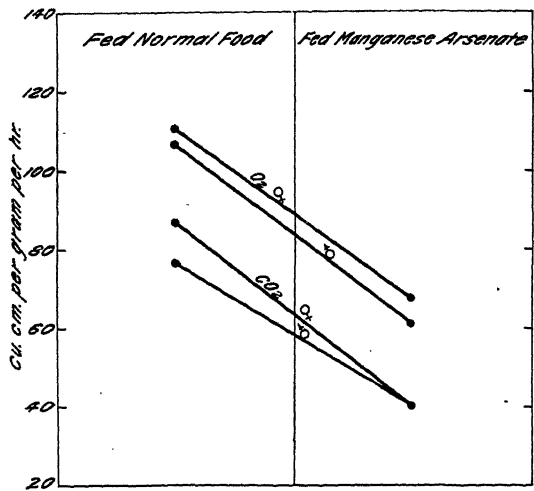


FIG. 3.—Normal rates of metabolism of potato beetles and effect of manganese arsenate on the oxygen intake and carbon dioxide output

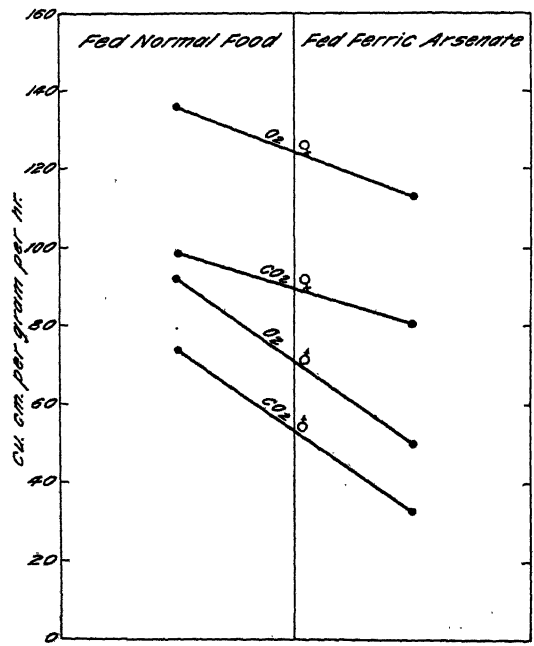


FIG. 4.—Normal rates of metabolism and effect of ferric arsenate on the oxygen intake and carbon dioxide output of potato beetles

likely that the divergence in the normal condition of the sexes as shown in Tables 1 and 2 was a primary factor in the reactions of these insects to ferric arsenate.

RESULTS WITH REALGAR (ARSENIC DISULPHIDE)

Realgar produced entirely different results from the arsenicals noted above. Since both the oxygen intake and CO_2 output of the beetles were increased during the first day, it would seem that realgar had a stimulating effect on metabolism. The average increase in the oxygen consumption of females was 9 per cent, in males 7 per cent. A decline of 13 per cent in the quotient in females occurred and a rise of 6 per cent in males (Tables 1 to 3 and fig. 5). Further proof of the stimulating effect of realgar is shown by the average increase in weight of 15 individuals, amounting to 0.9 per cent. The influence of realgar on metabolism when continued for a period of three days (fig. 6, E) shows that the respiratory exchange decreased profoundly during the last day.

The toxicity of an arsenical usually depends upon its solubility, the facility with which it can be absorbed by protoplasm, and the rate at which it can be excreted. From unpublished data on the solubility of arsenicals in the digestive tract of insects, it appears that the first stimulating effect of realgar on the respiratory exchange is attributable to its slight solubility and absorption by the protoplasm, and to its comparatively rapid rate of excretion. Further ingestion of realgar, however, finally brings about a cumulative effect and causes a depression of the gaseous exchange, as shown in Figure 6, E. This behavior of realgar may hold true also of any other arsenical which is ingested at first in very small quantities, or which like realgar has a comparatively low solubility and is rapidly excreted from the digestive system.

TABLE 5.—Average respiratory gas exchange of potato beetles fed the oxide component of arsenical compounds

Kind of feeding	O_2 intake	Probable error \pm	CO_2 output	Probable error \pm	$\frac{\text{CO}_2}{\text{O}_2}$	Probable error \pm	Per cent depression	
							O_2	CO_2
	<i>Cu. mm.</i>		<i>Cu. mm.</i>					
Normal.....	390	3. 21	305	2. 65	0. 782	0. 032	-----	-----
Lead oxide (with pipette).....	310	2. 15	246	1. 88	. 793	. 025	20	19
Normal.....	357	1. 72	217	1. 87	. 607	. 037	-----	-----
Calcium oxide (with pipette).....	337	1. 79	206	2. 16	. 611	. 042	5	5
Normal.....	320	3. 81	230	2. 11	. 718	. 063	-----	-----
Manganese oxide (with pipette).....	212	1. 86	189	3. 25	. 891	. 039	34	17
Normal.....	296	3. 26	193	2. 34	. 652	. 024	-----	-----
Ferric oxide.....	235	2. 46	147	1. 83	. 625	. 014	20	23

RESULTS WITH THE OXIDE COMPONENT OF ARSENICALS

The results of the tests with the oxide component of arsenical compounds are shown in Table 5 and in Figure 7. From Figure 7 it is readily noted that in every case a depression of the gaseous exchange occurred. With lead oxide (PbO) the depression amounted to 20 per cent in the oxygen consumption and 19 per cent in the CO_2 output; with manganese oxide (MnO) 34 and 17 per cent; with ferric oxide (Fe_2O_3) 20 and 23 per cent; with calcium oxide

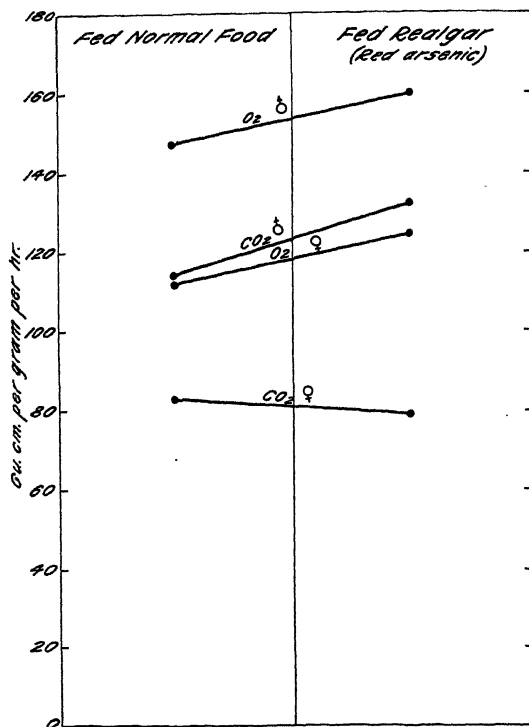


FIG. 5.—Normal rates of metabolism and effect of realgar on the oxygen intake and carbon dioxide output of potato beetles. Note the stimulating effect of feeding for one day and compare with Figure 6, E, where the beetles fed for 3 days

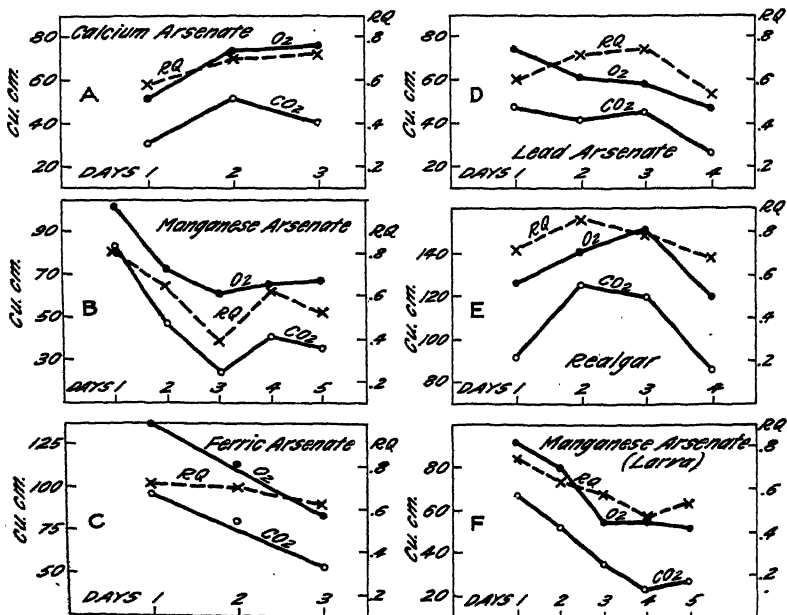


FIG. 6.—Effect on the metabolism of potato-beetle larvae and adults when feeding with various arsenicals was continued for several days

(CaO) an insignificant depression of only 5 per cent in both the oxygen consumption and the CO_2 production. It is quite evident from these results that the oxides possess some insecticidal qualities.

A slight increase in the respiratory quotient, especially with manganese and lead oxides, was obtained as a result of these tests.

RESULTS WITH ARSENIUS ACID (As_2O_3), ARSENIC ACID (As_2O_5),⁶ SODIUM ARSENITE (Na_2HAsO_3), AND SODIUM ARSENATE ($\text{Na}_2\text{AsO}_4 \cdot 12\text{H}_2\text{O}$)

The effect of arsenical compounds and of the oxide components on the respiratory metabolism of insects having been determined, a series of experiments with various dilutions of arsenious and arsenic acid were made to ascertain the effect that arsenic alone plays in depressing the gaseous exchange. After a large number of preliminary trials with dilutions of different concentrations, a neutral dilution of arsenious and arsenic acid was obtained that depressed the oxygen consumption to an equal degree with either substance. The concentration of arsenious acid per cubic centimeter was 0.00495 gm., and the concentration of arsenic acid per cubic centimeter was 0.0115 gm. These dilutions of arsenic were fed to the insects by means of a small graduated pipette, as previously described, which could deliver, by means of small drops of fluid flowing by gravity or slight mouth pressure, exactly 0.01 c. c. of solution. Other tests were included in which a small hypodermic syringe was used, and a definite quantity, that is, 0.01 c. c., was injected into the oral cavity. The objectionable feature of the syringe is that the forcing of the entire amount of liquid into the oral cavity causes the insects, in some instances, to regurgitate some of the injected fluid. Nevertheless, the results as a whole compare favorably with those obtained by means of the graduated pipette. The results of these experiments are averaged and summarized in Table 6. In Figure 8 the curves were drawn to show the effect of sodium arsenite and arsenate and arsenious and arsenic acids upon the respiratory metabolism of *Pheletes agonus*, *Leptinotarsa decemlineata*, and *Popillia japonica*. In the graphs of this figure the depression of the oxygen consumption is represented at hour intervals for the first 2 to 6 hours, and the effect on the oxygen intake 16 to 18 hours later is also shown. Utilizing the oxygen depression as a basis for judging the relative toxicity of the solutions on different species of insects, the graphs strikingly reveal the fact that the depression is more intense during the first few hours after the poison is consumed than at a later period. After this initial period of depression the lowering of the oxygen consumption continues gradually until a maximum is reached. This maximum of depression is utilized as a basis for comparing the relative toxicity of arsenious and arsenic acid.

⁶Anhydride arsenious and arsenic acids dissolved in water were used in these experiments, and for the sake of brevity and clearness are designated in the discussion as As_2O_3 and As_2O_5 , respectively.

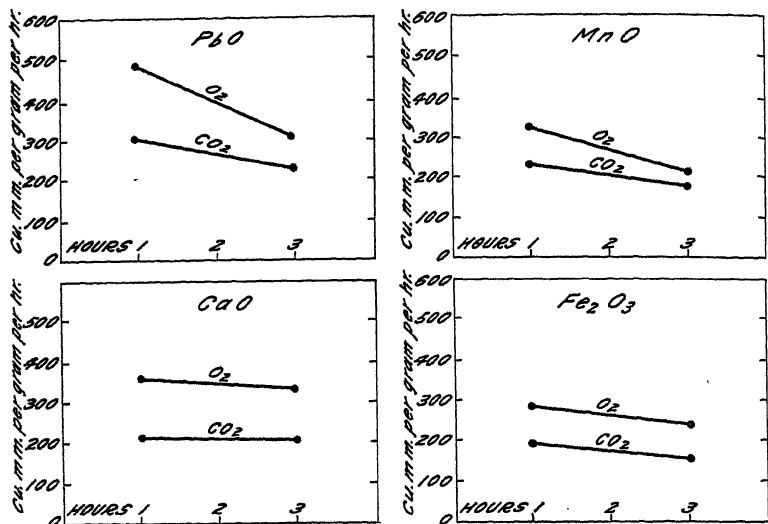


FIG. 7.—Normal rates of oxygen consumption and carbon dioxide output and the effect of 0.01 mgm. doses of lead oxide (PbO), calcium oxide (CaO), manganese oxide (MnO), and ferric oxide (Fe₂O₃) on the respiratory metabolism of potato beetles

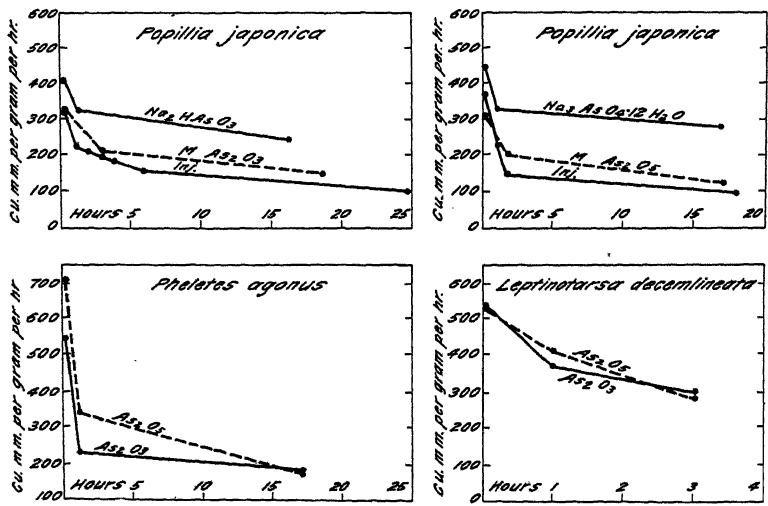


FIG. 8.—Effect of arsenious acid (As_2O_3), arsenic acid (As_2O_5), sodium arsenite (Na_2HASO_3), and sodium arsenate ($Na_2AsO_4 \cdot 12H_2O$) on *Leptinotarsa decemlineata*, *Phlebotomus agonus*, and *Papillia japonica*. The dotted line M represents feeding with pipette, the solid line Inj., feeding by injection in the oral cavity. This figure shows the oxygen inhibition at hour intervals. Note the intense depression which occurs during the first few hours as compared to that which occurs during the remaining period of the experiment

Since the concentrations of arsenious acid used were 0.00495 gm. per cubic centimeter, and of arsenic acid, 0.0115 gm. per cubic centimeter, the results show that the former was 57 per cent more toxic than the latter. Similarly, the curves representing the oxygen depression caused by sodium arsenite and sodium arsenate are practically identical in nature and reach the same level of depression (fig. 8). The concentration of sodium arsenite used was 0.0017 gm. per cubic centimeter, and the concentration of sodium arsenate was 0.00424 gm. per cubic centimeter. By calculating the relative toxicity of the two substances, it is found that the former is 59 per cent more toxic than the latter. Campbell (3) found that the arsenite was more toxic than the arsenate to the tent caterpillar, *Malacosoma americana* Fab.

A significant increase in the respiratory quotient was obtained as a result of these tests, as is shown in Table 6.

TABLE 6.—Average respiratory gas exchange of insects fed dilutions of arsenious acid (As_2O_3), arsenic acid (As_2O_5), sodium arsenite (Na_2HAsO_3), and sodium arsenate ($Na_3AsO_4 \cdot 12H_2O$)

Insect used	Kind of feeding	O ₂ intake	Probable error ±	CO ₂ output	Probable error ±	CO ₂ O ₂	Probable error ±	Per cent depression	
								O ₂	CO ₂
		<i>Cu.mm.</i>		<i>Cu.mm.</i>					
P. japonica.....	Normal.....	415	2.19	244	3.27	0.587	0.026		
Do.....	Na ₂ HAsO ₃ (injected).....	250	3.01	229	1.77	.916	.093	40	6
Do.....	Normal.....	446	1.83	185	1.59	.414	.018		
Do.....	Na ₃ AsO ₄ ·12H ₂ O (injected).	279	2.78	114	2.01	.408	.053	37	38
Do.....	Normal.....	322	4.52	221	3.72	.686	.068		
Do.....	As ₂ O ₃ (injected).....	150	3.06	141	1.57	.940	.042	53	36
Do.....	Normal.....	320	2.75	198	3.55	.618	.069		
Do.....	As ₂ O ₃ (pipette).....	115	2.81	97	2.04	.843	.042	64	51
Do.....	Normal.....	376	1.92	143	2.73	.380	.016		
Do.....	As ₂ O ₃ (injected).....	99	2.66	86	1.87	.868	.062	73	40
Do.....	Normal.....	315	3.66	253	3.42	.803	.063		
Do.....	As ₂ O ₃ (pipette).....	120	1.91	117	2.68	.975	.033	61	54
P. agonus.....	Normal.....	543	1.79	276	2.43	.508	.045		
Do.....	As ₂ O ₃ (injected).....	163	3.21	127	1.58	.779	.097	70	53
Do.....	Normal.....	708	2.07	287	2.34	.405	.041		
Do.....	As ₂ O ₃ (injected).....	171	1.89	123	3.42	.719	.044	75	57
L. decemlineata.....	Normal.....	550	1.76	321	4.13	.583	.035		
Do.....	As ₂ O ₃ (pipette).....	301	1.71	202	1.93	.671	.055	45	37
Do.....	Normal.....	546	2.36	341	1.59	.624	.027		
Do.....	As ₂ O ₃ (pipette).....	287	1.83	214	2.76	.745	.024	47	37

DISCUSSION

The evidence from a study of the data presented points to the general fact that arsenicals exert an inhibiting effect on the respiratory metabolism of insects. The significance of this inhibition lies in the fact that with young insects the metabolic activity is extremely more marked, and the reduction, therefore, more intense than with older ones. This would seem to indicate that the physiological activity of the living protoplasm is of prime importance in susceptibility to arsenical poisoning, since differences in susceptibility of groups of insects (young and old) correspond to differences in physiological age. In a previous investigation (8) it was pointed out that with the potato beetle the oxygen consumption and the carbon dioxide output decreased with advancing age and that a corresponding decline in the rate of oxidation through the inhibition of the enzymes

(oxidases and catalases) took place. Similarly, it would seem, a reduced metabolic activity as a result of arsenical feeding may be traced to the inactivation of oxidizing enzymes, which in some way prevents oxidative reactions. Shafer (12) called attention to the fact that certain volatile substances, such as gasoline and carbon disulphide, affect the oxidative and catalase activity of *Passalus cornutus* Fab. Voegtlin (13) suggested that the action of arsenic upon protoplasm is essentially due to an interference with the normal functioning of glutathione in the oxidation-reduction phenomena of tissues. The presence of glutathione has been detected by the writer in the species of insects mentioned in this paper. On evidence of the inhibiting action of arsenicals on the gaseous exchange mentioned above, it would appear that the toxic action of arsenicals is essentially due to an interference in cellular oxidations and reductions. If we inquire into the nature of susceptibility, whether or not it is in some way associated with or dependent upon the rate of oxidation in protoplasm, we again point to the fact that young insects showed a higher rate of oxidation and were more susceptible to arsenicals than older ones.

A chemical alteration of the protoplasm caused by the penetration of arsenic ions may render it more reactive or less reactive. From the data presented it is clear that, except in the case of realgar, the effect of the agents is to retard biochemical processes, the retardation caused by realgar occurring only after greater quantities of the poison have been ingested.

SUMMARY

The effect on the respiratory metabolism of potato beetles produced by feeding foliage sprayed with various arsenicals was studied. Experiments similar to those with potato beetles were made on wireworms by feeding grains of corn soaked in arsenical solutions.

The metabolic activity of potato beetles was reduced when they fed on lead arsenate, calcium arsenate, ferric arsenate, and manganese arsenate for 2 to 16 hours, and increased when they fed on realgar for the same period. The percentage depression of the oxygen consumption and the carbon dioxide production varied with the arsenical employed.

Realgar seemed to act as a stimulant at first, since both the oxygen intake and the carbon dioxide output increased and a gain in weight occurred. After three days' feeding, however, a depression of the gaseous exchange took place, indicating a cumulative action of this substance in the protoplasm.

Differences in susceptibility to lead arsenate appeared when sprayed foliage was fed to two groups of insects (young and old). In young insects the inhibiting influences were more profound. The inhibition exhibited by starving insects on the gaseous exchange was comparable to that of young insects.

The respiratory metabolism of insects was reduced when they were fed the oxide components of arsenical compounds; that is, lead oxide, manganese oxide, ferric oxide, and calcium oxide. The reduction with calcium oxide was, however, insignificant.

Arsenious acid, arsenic acid, sodium arsenite, and sodium arsenate depressed the oxygen consumption and the carbon dioxide production profoundly during the first few hours after insects were fed these solutions. The lowering of the oxygen intake continued gradually thereafter until a maximum depression was reached for the concentration of the solution. A significant increase in the respiratory quotient resulted from these tests.

With the concentration of arsenious and arsenic-acid solutions used it was found that arsenious acid was 57 per cent more toxic than arsenic acid. Similarly, sodium arsenite was found to be 59 per cent more toxic than sodium arsenate.

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A STEM-END AND CENTER ROT OF TOMATO CAUSED BY VARIOUS UNRELATED ORGANISMS¹

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INTRODUCTION

When an abundance of bacteria is found in plant tissue still in an early stage of disease, it is generally accepted that the disease has been produced by a specific organism. In the work of isolating the organism from the diseased material, should the platings yield pure cultures or nearly so, the belief is strengthened; and later if the original type of lesion is reproduced by inoculation, the worker is fully convinced that a specific germ is the cause of the disease. This is true as a generalization, but in the particular case with which this paper deals, more than one organism was found to be the cause of the typical diseased condition.

DESCRIPTION OF THE DISEASE

The disease in question is a rot of tomato fruits; it usually starts when the fruits are green and is scarcely noticed until they are full-sized or just beginning to turn. The decay is mostly internal and is hard to detect on the outside of the green fruit unless the stem end is examined very carefully. Here it shows as small brown spots or a thin brown ring around the stem end or a discoloration of the stem scar (fig. 1, A; fig. 2, A). There are cases where the surface of the fruit may not show any definite marks of disease, but usually if no dark spots or rings are visible at the stem end, the fruit at or before the pink stage of ripening takes on a sickly color. When it is cut open the placentae are found partly or wholly decayed. The diseased portion is hard and brown and if the fruit is pink or red the diseased area is almost black (fig. 1, B). The rot may extend inside from stem to blossom end, though usually it is confined to the upper half of the central placentae. No external sign of disease was seen at the blossom end of any fruits examined or reported on. Quite frequently the rot involves a portion of seeds, and these, together with the surrounding tissue, become a hard black mass. This mass can be separated intact from the surrounding tissue (fig. 2, E). The disease does not affect the shape of the fruit and there is no slime or ooze connected with it. Razor sections through the hard dark portions examined under the microscope showed motile bacteria in the cells and between them (?).²

Although the central decay is the most important feature of this disease, the rot at the stem end is not to be overlooked. This stem-end rot, however, is unlike that of the tomato stem-end rots hitherto

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² Reference is made by number (italic) to "Literature cited," p. 1023.

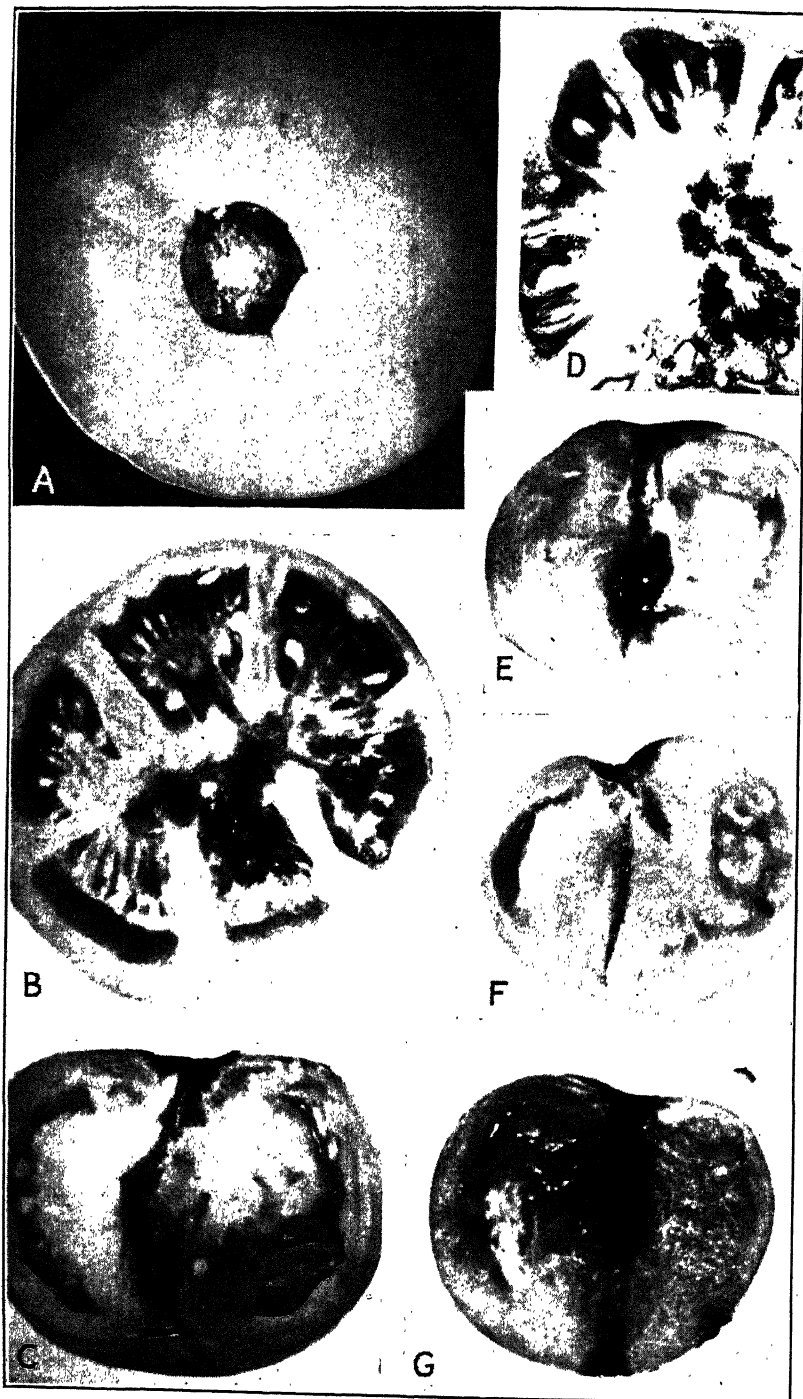


FIG. 1.—A, diseased tomato received from Texas in 1924. B, cross section of A. C, green tomato inoculated through stem end with the organism isolated from A; 4 weeks after inoculation. D, green tomato inoculated through stem end with the Texas organism; 5 days after inoculation. E, green tomato inoculated with *Bacillus coli*; 28 days after inoculation; infection slight. F, green tomato inoculated with *Bacterium citri*; 32 days after inoculation; infection slight. G, green tomato inoculated with *Bact. savastanoi*; 24 days after inoculation.

described. Of these one is a greenhouse rot caused by a *Botrytis* and the infection occurs on the calyx, fruit stalk, and stem end of either green or ripe fruit and advances into the fruit, softening it (4). Another is the *Phytophthora infestans* rot of tomatoes which is similar in its early stages on green fruit, but it attacks green or ripe fruit in any stage or size with the same ease, and the disease spreads beyond the stem in a broad dark area across the top of the fruit (5; 11; 13, p. 9-16). The fruit infection caused by *Cladosporium fulvum* Cke. is also more than a stem-end infection and spreads across the fruit, distorting and blackening the upper face of it. The hard central core is present, however (8).

ORIGIN OF THE DISEASE

The disease herein reported was first found in the tomato fields of Cherokee County, Tex. Since the fruit is shipped green, it was not observed until the tomatoes arrived at their destination and had changed to a pink color. The inspectors noticed the brown rings on the pink fruit and the unnatural shade of those still green and, cutting them open, found the decay well advanced through the central core (fig. 1, B).

There had been many light rains in this section accompanied by extreme heat. Some of the tomato growers attributed the disease to the weather conditions, others thought it might be due to an excess of sodium nitrate in the soil. Their losses from the disease were heavy; some days a third of the carloads arriving at market would be affected with the central decay.

In the summer of that year, 1924, the same disease occurred in Otoe County, Nebr., during the height of the shipping season (fig. 2, A). There were extreme changes in weather in the early summer; first it was very cold, then very hot, then too wet. In the late summer and early fall, when weather and growing conditions had altered, the disease became less virulent, and at the end of picking time the fields were nearly free from it.

Motile bacteria were found in the hard dark tissue of the Nebraska tomatoes, but no fungus was observed. In the region where the disease occurred in abundance there were some tomato growers with extended acreages who did not have a trace of it. They had not fertilized their soil at all or had turned under a cover crop and cultivated frequently during the growing period.

ISOLATIONS AND INOCULATIONS

The same bacterial organism was isolated from diseased fruit from both Texas and Nebraska, and the disease was reproduced by inoculating green tomatoes with bacteria from both isolations (fig. 1, C and D; fig. 2, B). The colonies on beef-agar plates were circular with entire margins, mostly smooth but some wrinkled (fig. 5, A and B). One-day-old colonies were cream colored in reflected light, but in transmitted light they had a broad cream-colored center with a blue area surrounding it. After a few days the entire colony was yellow.

The green fruits were first washed with mercuric chloride 1:1,000, then with sterile water, and a water suspension of the organism was smeared on with a soft sterile brush. Some of the tomatoes were punctured with a sterile needle at the stem and blossom ends, others

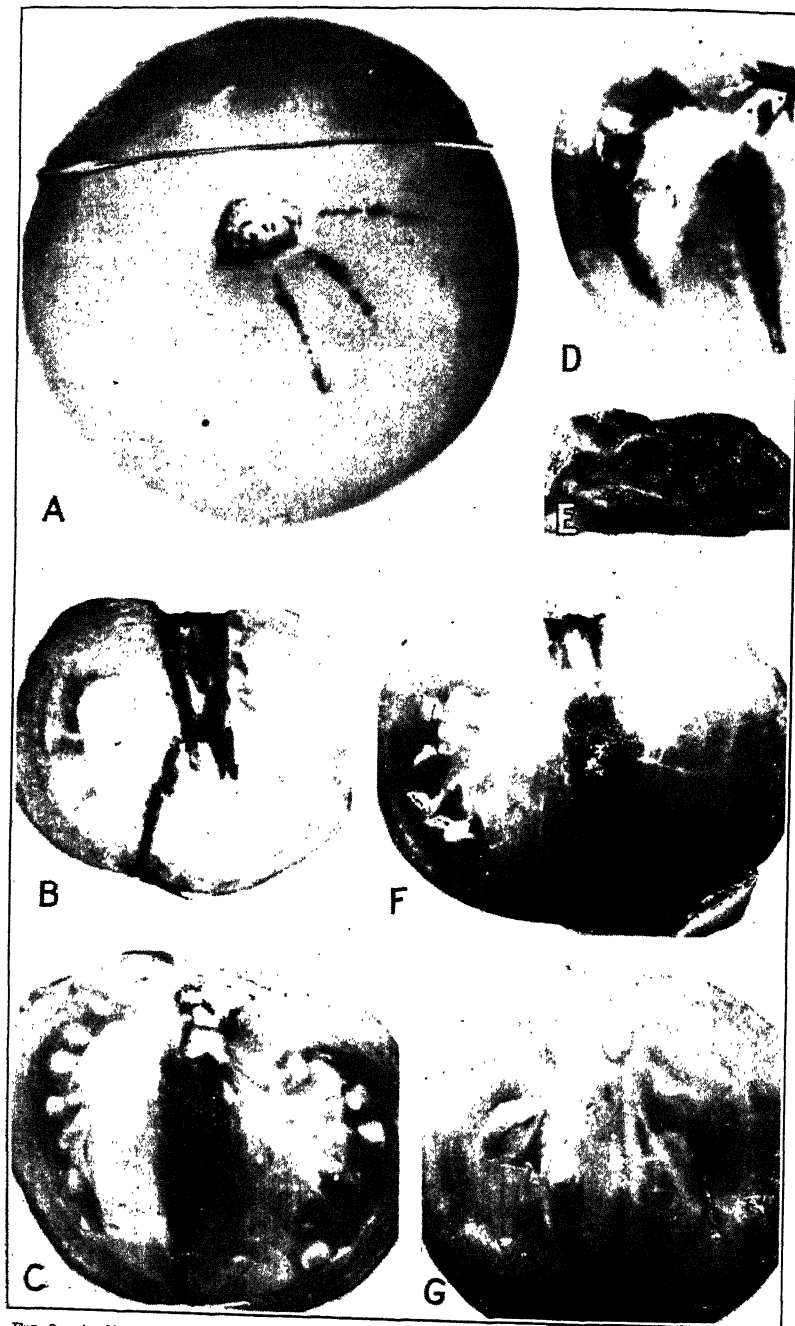


FIG. 2.—A, diseased tomato received from Nebraska in 1924. B, green tomato inoculated with organism isolated from A; 18 days after inoculation. C, green tomato inoculated with *Bacterium malvacearum*; 19 days after inoculation. D, green tomato inoculated with *Bacterium vitians*; 27 days after inoculation. E, hard central core removed intact from tomato inoculated 14 days before with the Nebraska organism. F, control tomato punctured without sterilizing, and infection followed; 4 weeks after puncturing. G, control tomato sterilized before puncturing; no infection after 4 weeks

were smeared and not punctured (fig. 3, C). Two hundred and fifty-six tomatoes attached to the vines were inoculated with the Texas and Nebraska isolations. About 95 per cent of the punctured and inoculated fruits became infected, whereas not more than 30 or 40 per cent of the smeared ones became infected. Inoculations were made in green fruit on the vines from 3 cm. in diameter to full size; infection occurred in all sizes. It required from 5 days to 4 weeks for a good infection to develop, and if the fruit ripened within 5 to 7 days after inoculation there was little or no infection. Where inoculations were made by smearing and not puncturing, it usually required more time for the infection to appear. The best infections occurred at greenhouse temperatures of 23° to 30° C. with plenty of moisture. Below 20° the infection was slight. Bacteria were found in the tissues of inoculated fruits (fig. 4, A).

Control fruits in a bed removed from the inoculated ones were washed with mercuric chloride, 1:1,000, then with sterile water, and punctured with a sterile needle. Only 2 out of 22 treated in this way became infected with the dark center rot, and one of these only slightly. Two others had soft rot and 18 remained perfectly sound (fig. 2, G).

For comparison 67 other tomatoes in the same bed were punctured *without washing* in mercuric chloride and sterile water. Thirty-eight became diseased with the hard, dark center rot in varying degrees (fig. 2, F); 10 had soft rot, and 19 remained sterile. A reference to these controls occurs later.

Inoculations were made in the leaves and stems of tomato plants, but no infection resulted. At first the stems browned somewhat, but in a few days the browning disappeared. Cabbage, cauliflower, and lettuce plants were also inoculated but with negative results. Green sweet peppers, however, became infected and hard, blackened masses of placenta and seeds resulted. The peppers were left to ripen before cutting and examining. There was no soft rotting. Bacteria were found in the cells of the dark portions.

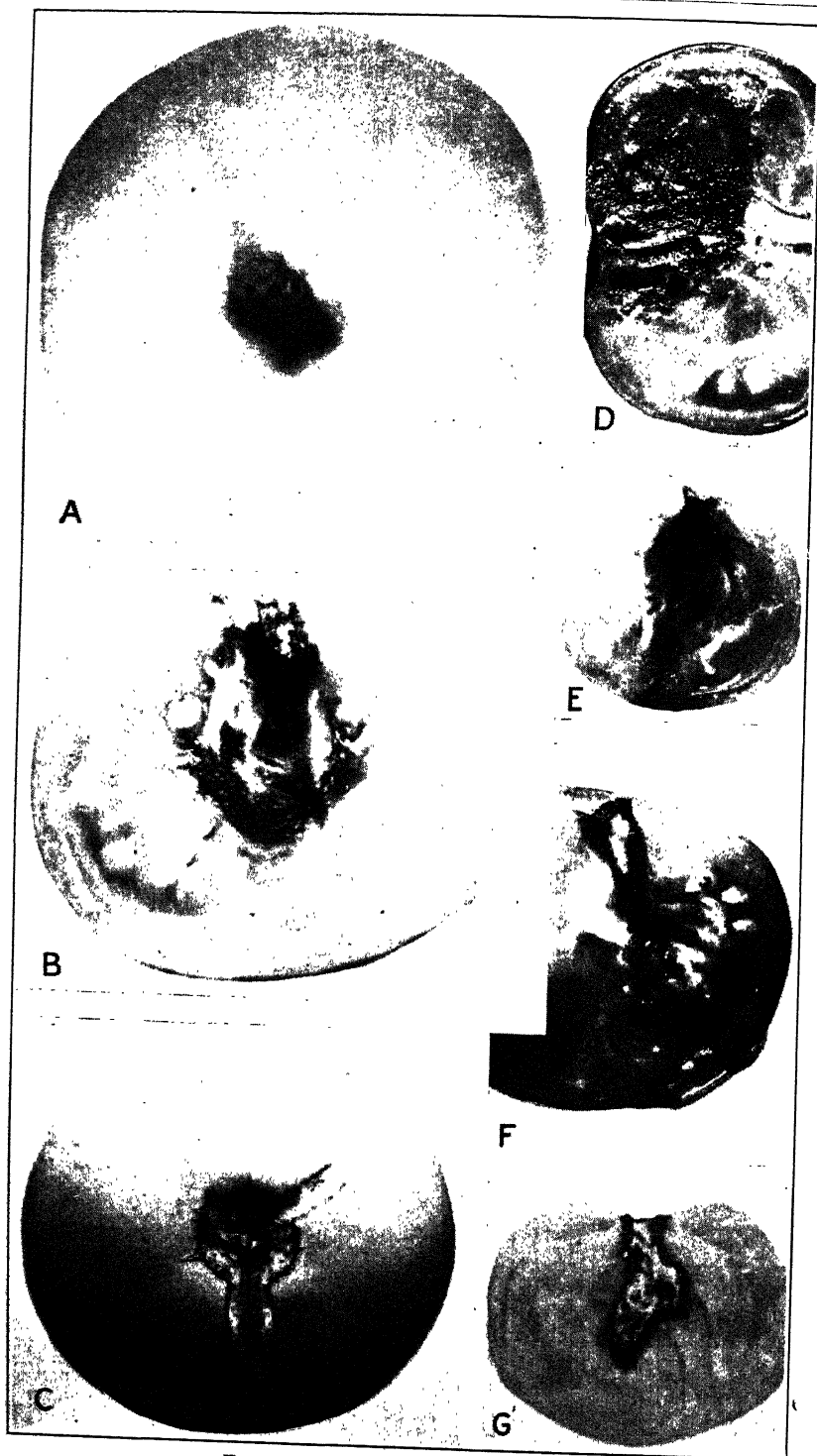
Water suspensions of the organism were smeared over tomato blossoms with a soft camel's-hair brush to determine if infections could occur at this stage of development. Out of 116 blossoms treated in this way which later formed fruits, only one was infected. Isolations were made from this fruit and the typical yellow organism was recovered. Inoculations made with subcultures of this organism established the pathogenicity of the reisolation.

This disease, therefore, can occur through infection at almost any stage from the blossom to the nearly mature but not ripe fruit. All inoculations were made with the fruit on the vines. The most favorable stage for infection seemed to be when the tomatoes were green and had not reached their full size but were swelling rapidly.

HYDROGEN-ION CONCENTRATION AND INFECTION

The hydrogen-ion concentration in tomatoes is higher in the ripe fruit than in the green. For the former the P_H has been found to be 4 and 4.2, while for the latter it is between 5 and 5.4 (?).

As the upper limit of hydrogen-ion tolerance of the Texas and Nebraska organisms in culture is 4.9 and its optimum P_H is around



5.5, it is easily seen why infection began readily in the green fruit and developed slowly, or did not occur at all if the fruit was nearing maturity and beginning to redden.

After the Texas and Nebraska strains had been grown in culture media for two years barely a trace of infection followed the introduction of the organisms into fruit which was nearing maturity. For two years they had been grown on stock media with the P_H varying from 6 to 7, and they could no longer accommodate themselves readily to the more acid medium of the nearly ripe tomato juice. There was not the same difficulty with the green tomatoes, although the infections were not so pronounced as those which occurred immediately after the first isolation.

PLATINGS FROM SEEDS OF TOMATOES INOCULATED WITH TEXAS-NEBRASKA ORGANISM

To determine whether seeds from diseased tomatoes can carry the infection, plates were poured from seeds in diseased fruits; not the darkened seeds, but those which looked to be sound. The seeds were sterilized 1 and 2 minutes in mercuric chloride, washed in sterile water, then crushed in beef bouillon, from which the plates were poured. The typical yellow colonies appeared on the plates.

Plates were also made from seeds which were sterilized and washed but not crushed. These seeds showed no discoloration but were taken from badly diseased fruits. They were immersed 1, 2, and 5 minutes in mercuric chloride and then washed in sterile water and allowed to stand in beef bouillon for 15 minutes before the plates were poured. The Texas-Nebraska colonies appeared on the plates poured from the seeds treated for 1 and 2 minutes, but there were none on those poured from the seeds treated for 5 minutes.

Although the organism can be carried by the seed, little fear need be entertained regarding the spread of the disease by this method, for it is not likely that seed would be saved from a tomato which had even a small amount of internal decay.

Seeds of the variety Bonnie Best of the sowing used in Nebraska which produced the disease were tested out to see if this particular lot had carried the disease. Four different sets of plates were poured from 450 seeds out of a pound. The seeds were crushed and soaked in beef bouillon one to three hours before the plates were poured in order to give ample time for the bacteria to ooze out or off of them. No colonies of this organism appeared on the plates. Evidently the disease had not been carried into Nebraska from seed produced elsewhere.

The year after the heavy infection in Nebraska the same seed beds were used without sterilizing, and no disease appeared. It was therefore obvious that the seed beds were not responsible for the occurrence of the disease.

EXPLANATORY LEGEND FOR FIGURE 3

A, blossom end of tomato inoculated with organism from Nebraska tomatoes; 19 days after inoculation. B, stem end of tomato inoculated with reisolation from A (blossom-end infection), giving typical stem-end infection; 25 days after inoculation. C, stem end of green tomato smeared with Texas organism; not punctured; 35 days after smearing. D, longitudinal section through A, showing blossom-end infection. E, green tomato inoculated with *Alternaria* sp.; 10 days after inoculation. F, tomato inoculated with *Bacterium viridilipidum*; 30 days after inoculation. G, green tomato inoculated with *Verticillium* sp.; 28 days after inoculation

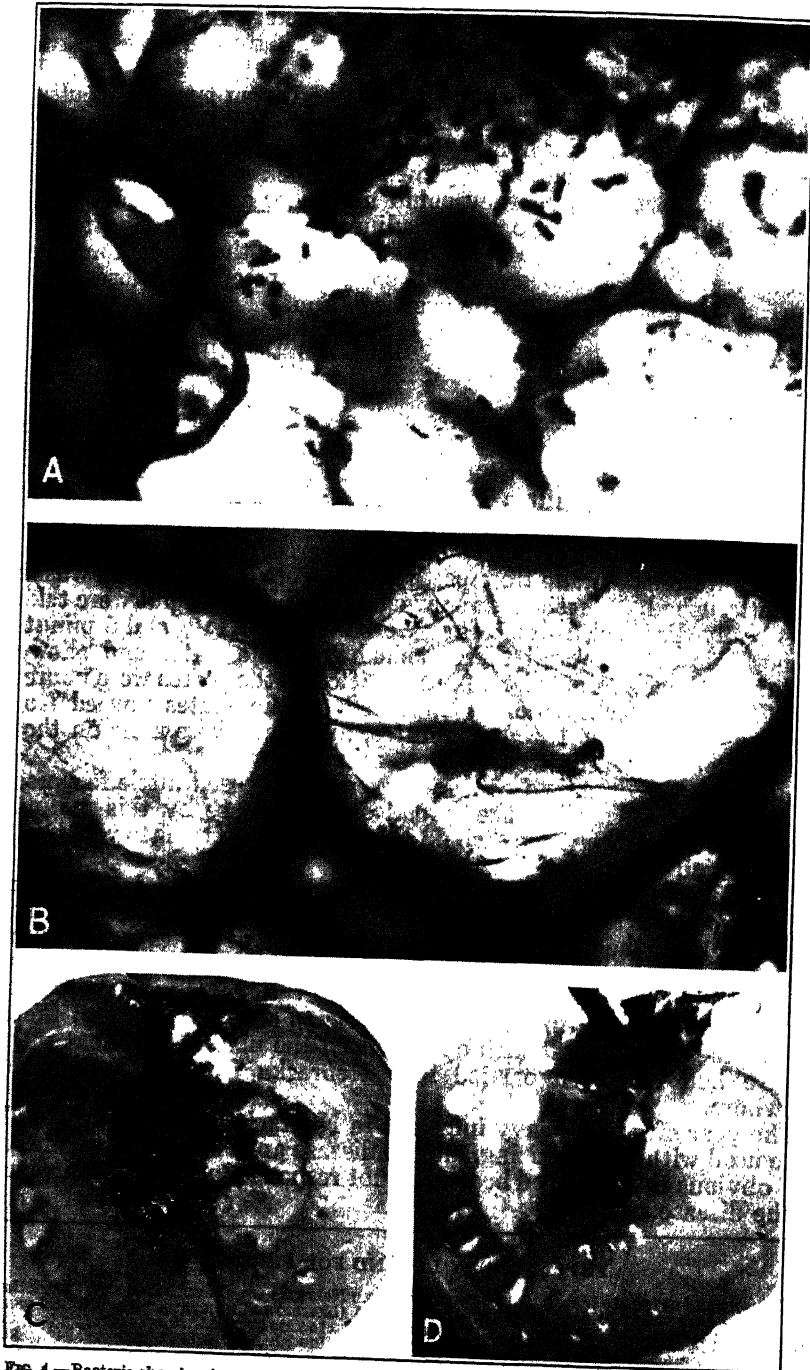


FIG. 4.—A, Bacteria showing in a stained section of tomato inoculated with the Nebraska organism; 18 days after inoculation; X about 1,500. B, mycelium of fungus showing in section of tomato inoculated with *Fusarium moniliforme*; 18 days after inoculation; X about 200. C, green tomato inoculated with *Gladosporium* sp.; 14 days after inoculation. D, green tomato inoculated with *F. moniliforme*; 14 days after inoculation; tomato very ripe and beginning to soften near calyx.

CULTURAL TESTS

Extensive cultural tests with colonies of the Texas and Nebraska isolations were made. The two proved to be similar organisms throughout nearly the whole of the series of tests as laid down in the descriptive chart of the American Society of Bacteriologists. In a test made with sugars for the production of gas one colony of the Texas isolation showed a variation. It was an active gas producer while the others were not. The purity of this colony was tested and established, and it was then used for further inoculations to see if by chance it would still produce the disease. It was as virulent as the other colonies and readily produced the characteristic lesions of the disease. So this colony was considered a variant, a thing which sometimes occurs with bacterial organisms. No other disturbing factor arose with the Texas and Nebraska strains.³

A short time after the cultural tests were completed some controls were made by puncturing tomatoes with a sterile needle but without first washing them with mercuric chloride and sterile water. Careful watch was kept on these, and in two weeks infection began to appear. Figure 2, F, shows the condition of one of them four weeks after puncturing. Razor sections through the hard central decayed part were examined under the microscope and bacteria were found as in the inoculated fruit. Platings were made, but instead of a pure culture of one organism two distinct types of yellow colonies were found, one of which was undoubtedly the Texas and Nebraska type which had been used for producing infections on fruits of neighboring plants but not in the same bed. For some reason instead of discarding the other yellow type as an intruder it was picked off, subcultures made of it, and later tomatoes were inoculated with it. This supposed intruder type also produced the disease after the usual time had elapsed, and the situation was thought rather confusing.

Shortly after this occurrence several untouched fruits in the neighborhood of some that had been inoculated with the Texas and Nebraska strains became infected with the same type of disease. These tomatoes were studied and only bacteria were found in the diseased parts. Isolation plates made from one of them gave two types of yellow colonies, one the Texas and Nebraska organism and another unlike it. This unknown type also produced the disease when green tomatoes were inoculated with it. When this second yellow unknown organism produced the typical central rot it was decided to try out a number of known pathogenic organisms and, much to the surprise of the writer, various ones produced the hard dark central lesions typical of the Texas and Nebraska disease.

TESTS WITH DIFFERENT ORGANISMS

It was decided to try first several yellow plant pathogens. The white colonies frequently found in association with the yellow ones on the plates had previously been tested for their ability to produce the hard central decay, but they had caused only a definite soft rotting. The yellow ones, on the other hand, had produced the typical hard central rot, as before stated.

³ The record of the cultural tests need not be given in this paper nor need the organism be named, since it proved eventually to be only one of several organisms able to produce the disease.

In every case before inoculating, the fruit was washed with mercuric chloride, 1:1,000, then with sterile water. Green fruit and some just before it turned red were inoculated and left on the vines to ripen.

Bacterium campestre (Pam.) EFS., the cause of the black rot of cabbage and other crucifers, produced very good infections as did *Bact. marginale* Brown (fig. 5, F) and *Bact. viridilividum* Brown (fig. 3, F), the cause of a leaf spot and rot of lettuce. Reisolation plates were made from the "marginale" and "campestre" tomatoes and the organisms were recovered.

Bacterium citri Hasse (fig. 1 F), the organism producing citrus canker, gave a few good infections and some slight ones, but for the most part the results were negative. Conditions evidently had to be very favorable for this organism. The same was true of *Bact. vitians* Brown (fig. 2, D), an organism causing a stem rot and leaf spot of lettuce.

Bacterium malvacearum EFS. (fig. 2, C), the cause of a leaf spot of cotton, produced the characteristic hard centers, but they were only occasionally dark colored.

Bacterium savastanoi EFS. (fig. 1, G), an organism which produces tubercles on the olive tree, produced a very good hard central decay of tomatoes.

Bacterium gummisudans McCulloch, which causes a leaf spot of *Galdiolus*, did not produce the disease. It was tested under various growth and temperature conditions, but the results were always negative.

Aplanobacter michiganense EFS., which produces a wilt of the tomato stem and a yellowing of the fruit, did not induce any hardening or browning of the central tissue.

The well-known colon bacillus, *Bacillus coli communis* Esch., was able to produce a slight infection in 2 cases out of 15 (fig. 1, E). *B. mycoides* Flüge, a common soil organism, gave negative results in all but 5 out of 19 inoculations.

At the conclusion of the experiments with the yellow organisms several white pathogens were tested.

Bacillus tracheiphilus EFS. did not produce the disease. It was tried at different seasons and under different temperature and moisture conditions.

Two strains of *Bacterium tumefaciens* Smith and Townsend, which are common in the soil, were tried. One was isolated from the hop gall and another from the peach gall. The hop strain produced the typical tomato lesions very quickly, but no galls formed (fig. 5, E). The organism was reisolated, and on the reisolation plates the usual smooth, round colony was found somewhat changed by passing through this host, and appeared as a wrinkled and humpy one (fig. 5, C and D). Subcultures of it produced good galls on *Ricinus*, but none on geranium or tomato stems, as the original and typical crown-gall hop strain does. The tomato stem was browned for more than 3 cm. on either side of the inoculation point but not galled. The peach strain of *Bact. tumefaciens* also produced the typical hard brown internal decay in the tomato fruit (fig. 5, G). Altogether, 35 green tomatoes were inoculated with *Bact. tumefaciens*; 16 with the hop strain and 19 with the peach strain. The hard brown centers resulted in all but two. These two remained sound and healthy.

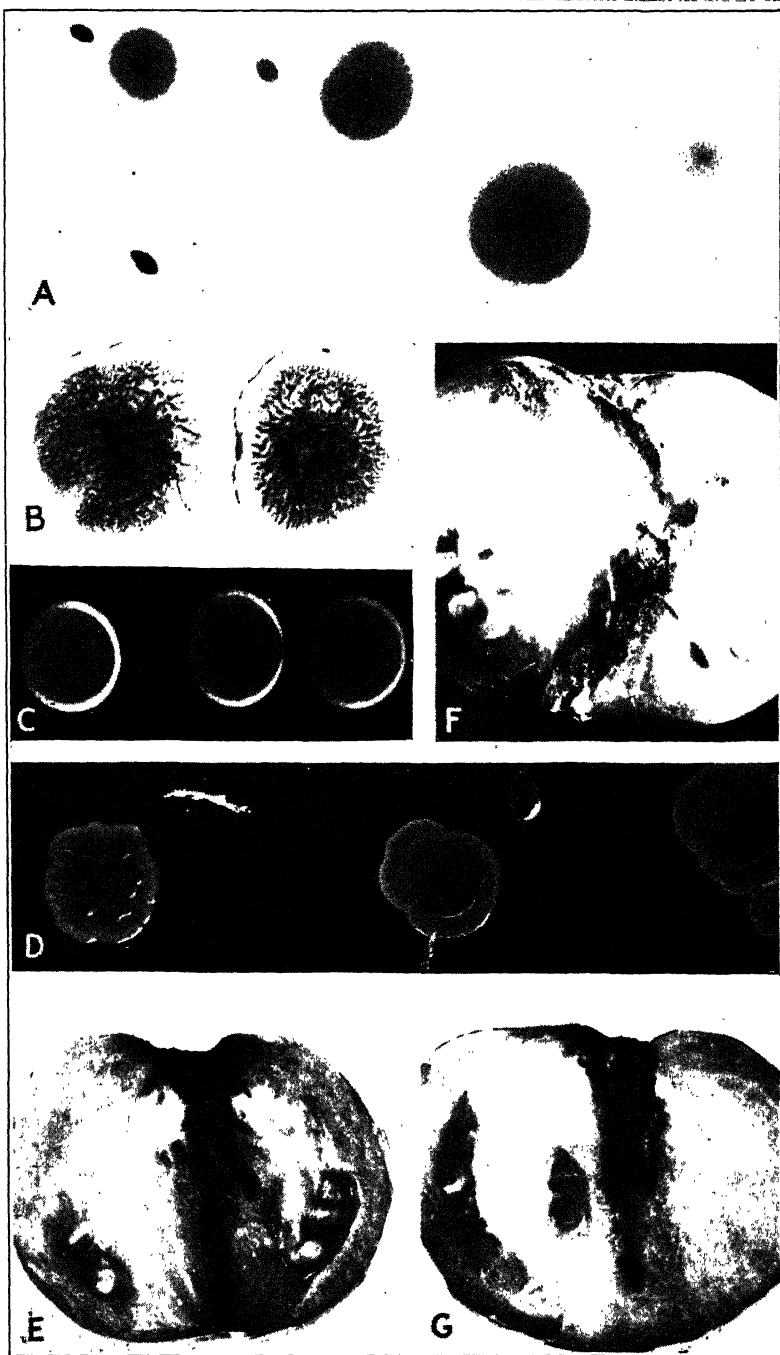


FIG. 5.—A, colonies of the Nebraska-Texas organism on beef-agar plates, enlarged about five times; smooth type; infectious. B, wrinkled type of Nebraska-Texas organisms on beef-agar plates, enlarged about five times; this type also infectious. C, typical colonies of *Bacterium tumefaciens*, hop strain on beef-agar plates; enlarged about five times. D, wrinkled type of *Bact. tumefaciens* colonies; hop strain on beef-agar plates; wrinkled character produced by passage of the strain through tomato fruit. E, green tomato inoculated with hop strain of *Bact. tumefaciens*; 18 days after inoculation. F, green tomato inoculated with *Bact. marginale*; 30 days after inoculation. G, green tomato inoculated with peach strain of *Bact. tumefaciens*; 18 days after inoculation.

A few fungi were tested in the same way. A *Verticillium* sp. causing a wilt of tomato stems, and another *Verticillium* causing a wilt of heliotrope (fig. 3, G.) produced the hard, dark core without any softening of the tissue. The fruit was always left on the plants to ripen before the examination inside was made.

An *Alternaria* isolated from stems of *Rosa hugonis* was tried and found to produce the hard, dark central rot (fig. 3, E). The fungus was recovered on reisolation plates.

An *Alternaria* has been noticed before in connection with a tomato central rot. A few years ago F. J. Pritchard examined lesions of this sort and found a fungus associated with it which he identified as an *Alternaria*. He was unable at the time to pursue the investigation. Several summers later he received similar diseased tomatoes from Nebraska and found bacteria in the cells, but no fungus. He concluded that the *Alternaria* which he had found previously must have been a secondary organism, and that he had overlooked the bacteria. The writer received the Nebraska material from him, and it was the bacterial organism isolated from these tomatoes which proved to be similar to the one isolated from the Texas tomatoes.

A *Cladosporium* sp. pathogenic to cranberry fruit produced the typical lesions in 25 out of 30 inoculated green tomato fruits. The color was almost black (fig. 4, C). The fungus was reisolated.

Septoria gladioli Pass., which produces spotting of leaves and dry rot of corms, gave negative results when inoculations were made in the winter. In the spring, however, the conditions were more favorable, and the broad, hard central core resulted. There was not the very dark color, however, which accompanied the *Verticillium* and *Cladosporium* infections.

Fusarium moniliforme Sheldon, a fungus which produces a soft rot of the Smyrna fig, also produced the typical lesions, and when the tomatoes were absolutely ripe there was also a soft rotting. The tissue in some parts of the tomato just below the epidermis became watery looking, and microscopic examination showed not the presence of a soft-rot bacterial organism but an abundance of mycelium and the curved septate spores of *F. moniliforme* (fig. 4, B). If the tomatoes were cut when red, but not perfectly ripe, only the central hard decay was present (fig. 4, D). The *Fusarium* was readily reisolated.

The hard central core produced by these various fungi could be lifted out intact, as in those fruits inoculated with bacteria. The texture of the core produced by a fungus sometimes had a leather-hardness instead of the crystallike or woody hardness produced by the bacteria. Occasionally small hollow spaces occurred in the central core, and the leathery texture was probably produced by these hollows being filled with a mycelial mass. In the tomatoes inoculated with bacteria these spaces were rarely present.

From the tests, it is seen that out of 13 different known bacterial organisms introduced into tomatoes in the same way and under the same conditions as the original Texas and Nebraska isolations, 6 produced positive results; results typical of those obtained by inoculation with the Texas and Nebraska strains. Four other organisms gave positive results under very favorable conditions, and three gave negative results. Of the five fungi tested all produced similar disease, and one produced a soft rot in addition.

The number of bacteria and fungi tested in this way might be extended indefinitely. Enough have been worked with, however, to establish the necessary proof that the stem and central decay of tomatoes is a parasitic disease induced while the fruit is green by various organisms under conditions of high temperature and high moisture. Those bacterial organisms which gave negative results and those which gave positive results only occasionally might, under certain conditions, be as positive as the Texas and Nebraska organisms themselves. The tomato varieties used in this work were Livingston's Globe and Bonnie Best. Both seemed equally susceptible. It is possible that another variety of tomato might have been favorable to the production of the disease if inoculated with those organisms which gave negative results with the above-named varieties. Certain fertilizers, too, might influence infection; so that it could be induced by those organisms which in the experiments with Bonnie Best and Livingston's Globe had proved noninfectious.

There was very little trouble from bacterial soft-rot organisms getting into the punctures. The careful washing of the tomatoes with mercuric chloride and sterile water before inoculation evidently prevented this from happening. The controls so treated were a striking evidence of the slight extent to which it occurred. Had the work been done out of doors more secondary infection might have developed.

ENTRANCE OF ORGANISMS INTO THE TOMATO

It is likely that the attacking organisms made their entrance at the region where the cuticle ends and the stem begins, for it was there that the black ring occurred.

The warm weather and excessive moisture in Texas and Nebraska undoubtedly produced rapid growth in the tomatoes, resulting in tiny breaks in the tissue at the cuticle margin, through which surface organisms can enter. In some instances the rapid growth might have stretched the cuticle at its juncture with the torus, thinning it so that the entrance to an active organism was made easy. In other cases it may have been that raindrops persisted at this juncture area long enough to injure some of the cells, and a slight extrusion of cell content could easily furnish enough food for the bacteria to begin their activities.

The stomata of the calyxes are another avenue of entrance to germs. From the calyx a passage can easily be gained to the torus and thence into the fruit. The calyxes of many inoculated fruits were examined and bacteria were found in them, causing first a watery appearance and in a later stage a hard, dry one.

OBSERVATIONS ABOUT BLOSSOM-END ROT OF TOMATOES

It seems to the writer that the same conditions which produce the stem-end and center rot of tomato may be responsible for the blossom-end rot, which of late years has been classified as a nonparasitic disease, the cause being laid to the breakdown of the tissue due to irregularities in the water supply; the bacteria and fungi which are present being considered secondary (1). The blossom-end rot has been studied by pathologists extensively in different countries and bacterial or fungous organisms have always been found associated with it (2 3 4 5 6 7 8 9 10 11).

Figure 3, A and D, shows an inoculation at the blossom end of a tomato with the bacterium isolated from Nebraska tomatoes which were affected at the stem end and center. The appearance is not unlike that of blossom-end rot. To establish further evidence that this condition at the blossom end was really produced by the Nebraska germ with which it was inoculated, isolation plates were made and the organism was recovered. The pathogenicity of the recovered germ was further tested and proved by inoculations into the stem end of other tomatoes (fig. 3, B).

GENERAL PREVENTIVE AND CONTROL MEASURES

As both soil and weather conditions are responsible for the presence of the stem-end and center rot of tomatoes in a field, the grower may be able to prevent or lessen it very materially by using manure which has passed through all the active stages of disintegration. This will assure him of fewer active soil organisms on his fruit and consequently of less disease should the weather conditions be favorable for them to get into it. Cover crops should be turned under at least a month before planting.

If an uninterrupted growth of tomatoes can be kept up during the fruiting season there will be little chance of the disease making any headway. A period of hot, dry weather holds back the growth of the plant, necessarily retarding the growth of the outer part of the fruit. Then if wet weather comes and rapid growth follows, the fruit fills up with water, and as the epidermis can not keep pace with the swelling cells inside, tiny cracks result. Frequent cultivation will keep the plants growing and prevent the fissures from forming. Evidence of this was very striking in Nebraska, where well-cultivated fields had practically no diseased fruit at the time of the heavy infection of other fields which were cultivated only sparingly.

The grower should also be careful to select varieties which are known not to crack easily.

As the disease can be produced by various bacterial and fungous organisms, it is highly essential to have clean seed, and any seeds that are slightly discolored or undersized should be avoided. Sanitary field methods should be followed.

The use of Bordeaux mixture is considered an effective germicide for tomato diseases. If the bacterial and fungous flora of the surface of green tomatoes is reduced or weakened by frequent spraying with this mixture there will be very little chance of the disease occurring.

SUMMARY

A stem-end and center decay has been responsible for heavy losses in shipments of green tomatoes. The disease can scarcely be detected on the outside of the fruit, while the central core, including portions of the seeds, is a mass of hard, dark tissue.

The disease occurs usually during seasons of hot weather and abundant rain, or during weather in which the fruit expands too rapidly after a period of checked growth. There is always an organism present which is responsible for the decay. It may be either a bacterium or a fungus. In a given location it may be a certain organism active and prevalent in that particular soil, whereas in another location it may be a different and entirely unrelated organism.

The work reported in this paper began with a yellow bacterial organism isolated from tomatoes similarly diseased in Texas and Nebraska in 1924. This organism was considered the specific cause of the disease for over a year. Since then, six different bacterial and five different fungous organisms have been inoculated into green tomatoes, and each has readily produced the stem-end and center rot. Four other bacterial organisms produced the lesions only after repeated trials and when conditions happened to be favorable to them. The results with three others were always negative.

The organisms which produced the decay readily were *Bacterium malvacearum* EFS.; *Bact. marginale* Brown; *Bact. tumefaciens* Smith and Townsend; *Bact. savastanoi* EFS.; *Bact. campestre* EFS.; *Bact. viridilividum* Brown; *Cladosporium* sp.; *Septoria gladioli* Pass.; *Verticillium* (two species); *Fusarium moniliforme* Sheldon; and *Alternaria* sp. The four organisms which produced the disease with difficulty were *Bact. citri* Hasse; *Bact. vitians* Brown; *Bacillus coli communis* Esch.; and *B. mycoides* Flügge.

The organisms which failed to produce the disease were *Bacillus tracheiphilus* EFS., *Bacterium gummisudans* McCulloch, and *Aplanobacter michiganense* EFS.

It is likely that there are many other bacteria and fungi able to produce the same lesions in tomatoes given the right conditions for development. The acidity of the fruit and proper conditions of growth are probably factors in preserving it from many of the organisms present in richly fertilized soil.

Blossom-end rot of tomatoes, considered by pathologists to be a physiological disease induced by irregularities in the water supply and not by parasites, was produced by inoculating tomatoes with the Texas-Nebraska organism. As the stem-end and center rot can be produced on growing green tomatoes by various bacteria and fungi when temperatures are high and moisture is abundant, it is quite possible that the blossom-end rot is produced in the field in the same way.

Preventive measures may be taken by using varieties which do not crack readily, fertilizing with well-seasoned manure in which the organisms are less active, cultivating the soil frequently, using sanitary methods in the field, and spraying with Bordeaux mixture.

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THE RELATION OF BLACK ROT TO THE STORAGE OF CARROTS¹

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INTRODUCTION

The black rot of carrot (*Daucus carota* L.) caused by *Alternaria radicina* M. D. and E., was discovered in 1918 by Meier, Drechsler, and Eddy on Long Island, N. Y., and by the writer in Washington, D. C. Meier, Drechsler, and Eddy discovered the causal organism produced the disease on the roots and foliage, and found that infection of roots in storage resulted from infection of the foliage. A description of the disease and excellent drawings of the fungus have been published.²

The disease as it occurs on the roots of carrot is of considerable economic importance. It has been observed on the New York and Washington markets nearly every season since its discovery. No accurate survey has been made of the extent of the loss caused by this rot, but the following example illustrates how serious it may become. A half ton of carrots (Danvers Half-Long variety) was purchased on the Washington market in November, 1921, and stored at temperatures ranging from 0° to 2° C. at Arlington Experiment Farm, Rosslyn, Va. The carrots were grown at Williamson, N. Y., shipped to Washington in bags in a carload lot, and removed to storage soon after arrival. At the time of storing they were in excellent condition and no black rot was observed. Two months later, however, the disease became evident. On May 20, 1922, after 185 days in storage, four crates were selected at random and a count was made of the diseased and healthy roots. Three hundred and two out of 488 carrots, or 62 per cent, had black rot (Table 1). The 62 per cent did not necessarily represent a total loss, for it included all infected roots no matter how few or how small the lesions. Roots with few and small lesions can be utilized, but since they are marred in appearance, their market value is of course reduced.

The decay caused by *Alternaria radicina* represents only a part of the loss that may result from the presence of the disease, for black-rot lesions afford a favorable opportunity for the entrance of other fungi such as *Sclerotinia* and *Botrytis*. *Botrytis* especially is dependent on some such favorable means of entrance. Blackman and Welsford³ have shown that this fungus in order to attack the leaves of *Vicia faba* L. readily must have a start on dead organic matter. Such a start is conducive if not indispensable to infection of carrots by *Botrytis*. The usual method of infection is through dead root tissue (the root tip, rootlets, and the crowns.) The black-rot lesions often crack and leave dead carrot tissue exposed to the action of fungi that

¹ Received for publication July 3, 1926; issued December, 1926.

² MEIER, F. C., DRECHSLER, C., and EDDY, E. D. BLACK ROT OF CARROTS CAUSED BY *ALTERNARIA RADICINA* N. SP. *Phytopathology*. 12: 159-166, illus. 1922.

³ BLACKMAN, V. H., and WELSFORD, E. J. STUDIES IN THE PHYSIOLOGY OF PARASITISM. II. INFECTION BY *BOTRYTIS CINEREA*. *Ann. Bot. [London]* 30: 389-398, illus. 1916.

may be present. Botrytis always occurs to some extent in carrots under storage conditions, and roots kept for long periods at temperatures ranging from 0° to 5° C. invariably become infected with it. The decay caused by this fungus is much greater in carrots affected with black rot than in those not so diseased.

TABLE 1.—Amount of infection that developed during storage on the roots of carrots when the foliage was inoculated with *Alternaria radicina* ^a

Where carrots were grown	Season	Treatment of carrots	Duration of storage in days	Number of roots stored	Number of roots infected	Percentage of roots infected
U. S. Department of Agriculture grounds.	1920-21	Foliage inoculated.....	215	414	412	99.5
Arlington Experiment Farm.		Foliage not inoculated.....	215	448	260	58.0
U. S. Department of Agriculture grounds.	1921-22	Foliage inoculated.....	201	341	152	44.6
Arlington Experiment Farm.		Foliage not inoculated.....	201	331	8	2.4
Williamson, N. Y.	1921-22	do.....	201	2,360	5	.2
U. S. Department of Agriculture grounds.		do.....	185	488	302	62.0
U. S. Department of Agriculture grounds.	1922-23	Foliage inoculated.....	120	426	422	99.0
Arlington Experiment Farm.		Foliage not inoculated.....	120	144	19	13.2
		do.....	120	151	1	.6

^a Storage temperatures ranged from 0° to 2° C. in each experiment.

DESCRIPTION OF THE DISEASE

ON THE LEAVES

There is little to add to the previous description of the disease as it occurs on the leaves.⁴ The resemblance of the symptoms of the black-rot disease to those of carrot blight (*Macrosporium carotae* E. and L.) might be emphasized, especially during the final stages when the outer leaves are killed. The writer has been unable to distinguish from macroscopic examination leaves killed by *Alternaria radicina* and *M. carotae*. The two diseases resemble each other in that there is a tendency for the outer leaves to be killed, but differ in that the blight lesions are more widely distributed over the foliage than are those produced by the black-rot organism. During blight epiphytotic the entire tops are often killed.

ON THE ROOTS

The lesions as they occur on the sides of the roots are circular to irregular in outline, slightly depressed, usually shallow, penetrating from 1 to 3 mm. deep (fig. 1). When infection occurs at the crown, the decay generally penetrates rather deeply, particularly into the core.⁵ As a rule, the color of the decay is almost jet black, but sometimes it is a greenish black. The black fungous growth may or may not occur on the surface of the lesions.

ORIGIN OF THE DISEASE ON THE ROOTS

PLACE OF STORAGE

The storage house may be a source of the disease if it has been used for the storage of diseased roots. Uncontaminated and healthy roots do not become diseased if stored in a house that has not been contaminated by the previous storage of diseased roots. It would seem, therefore, that the disease has its origin in the field.

⁴ MEIER, F. C., DRECHSLER, C., and EDDY, E. D. BLACK ROT OF CAROTS CAUSED BY *ALTERNARIA RADICINA* N. SP. *Phytopathology* 12: 159-166, illus. 1922.

⁵ MEIER, F. C., DRECHSLER, C., and EDDY, E. D. Op. cit.

DISEASED FOLIAGE

Evidence has been submitted to show that infection of the roots in storage results from infection of the foliage.⁶ The following experiments, conducted during three seasons, substantiate this evidence.

The carrots used in these experiments were grown in the grounds of the United States Department of Agriculture at Washington, D. C., on a small piece of ground suitable for carrot culture, but probably never employed for that purpose. They were planted between the first and twentieth of June each season. The ground was divided into two plots, one of which was inoculated and the other held as a check. The foliage was inoculated by thoroughly spraying it with a spore suspension of *Alternaria radicina* in humid weather during late August or early September⁷. For this purpose an atomizer was used.

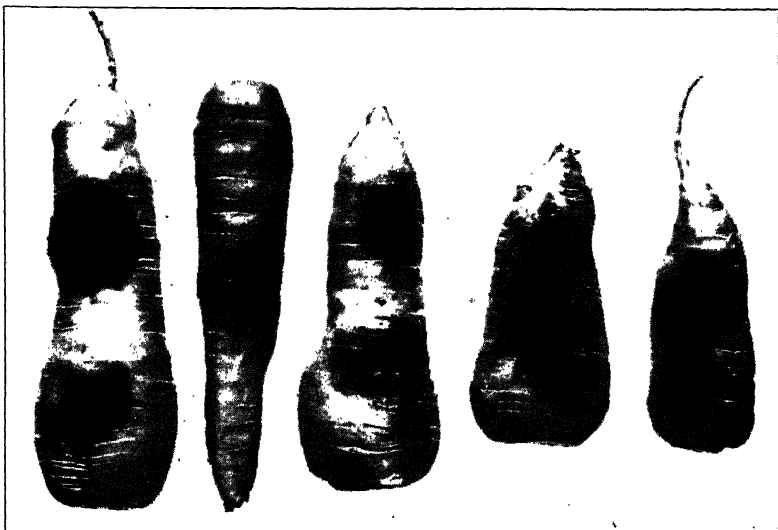


FIG. 1.—Typical lesions of black rot as they occur on the sides of carrot roots

A pathological condition of the foliage resulted from the inoculations very similar⁸ to that produced by *Macrosporium carotae*, which was also present to some extent each season. The disease spread after a time to the checks, but here it never became as severe as in the inoculated plot.

Diseased leaves from these plots were examined in the laboratory from time to time each season. *Alternaria radicina* was found on many of the lesions and was isolated a number of times from diseased tissue, usually from the lower leaves. *Macrosporium carotae* was occasionally obtained instead of *A. radicina*.

The roots were dug and stored in early November, and no disease was observed on them at that time. They were again examined at the end of the storage period (April and May). The results are

⁶ MEIER, F. C., DRECHSLER, C., and EDDY, E. D. Op. cit.

⁷ 1920. Aug. 25, day of inoculation, sky cloudy; Aug. 26, heavy fog during morning, settling down to earth, foliage wet, drops of water on all the foliage; Aug. 27, fog during morning and rain during night.

1921. Aug. 27, day of inoculation, rained night before and up to time of inoculation; cloudy all day and most of following night.

1922. September between seventh and fifteenth; cloudy during two days following inoculation.

⁸ MEIER, F. C., DRECHSLER, C., and EDDY, E. D. Op. cit.

recorded in Table 1. Table 1 also contains data on carrots grown at Arlington Experiment Farm, Rosslyn, Va., and carrots grown at Williamson, N. Y. which were stored under the same conditions (temperature 0° to 2° C.) as those from the inoculated and check plots.

An examination of Table 1 reveals the fact that there was an abundance of black rot in roots from inoculated foliage and a liberal amount in roots from the check, showing again that the disease spread from the inoculated to the check plot. A trace of black rot was found in stored carrots grown at Arlington Experiment Farm following the storage of diseased roots in the same room. It is believed that this trace had its origin in these diseased roots.

The foregoing data would indicate that diseased foliage is a source of root infection were it not for the fact that the foliage disease has never been observed to occur naturally. In 1919 a careful examination was made of plants growing in fields where carrots which developed black rot had grown the previous year⁹ but no evidence of disease could be found. Several attempts have since been made to find the foliage disease but without success. The disease of the roots on the other hand, has been observed nearly every season since it was discovered. The contingency of the root disease upon the foliage disease must therefore be subject to question until the latter is observed to occur naturally.

INFECTED SOIL

The data recorded in Table 2 prove that it is not necessary for the disease to pass directly through foliage into the roots in order for them to become infected. These data were obtained from two of the experiments discussed on page 1027. The foliage was inoculated in late August; the roots were harvested in early November and stored at temperatures ranging from 0° to 2° C. until June the first season and until April the second. It will be seen from the data that more infections occurred at the sides of the roots than at the crowns. It is also possible that some of the infections at the crown occurred independently of the infection of the foliage. It is just as conceivable that the infections of the roots, and especially those on the sides, might originate from a contaminated soil as from a diseased foliage.

TABLE 2.—Points on the roots of carrots at which infection produced by *Alternaria radicina* occurred.

Season	Number of roots infected on—					
	Crown	Side	Crown and side	Crown and tip	Crown, tip, and side	Tip and side
1921 and 1922.....	32	179	89	5	247	60
1922 and 1923.....	11	17	67			

Alternaria radicina is a vigorous saprophyte and will live indefinitely on dead organic matter. It is not unreasonable, therefore, to believe that it may live in the soil, and thus the soil may be a source of inoculum for the infection of the roots. Experiments are under way to determine whether or not soil may be a source of root infection.

⁹ MEIER, F. C., DRECHSLER, C., and EDDY, E. D. Op. cit.

TABLE 3.—Infection of carrots by *Alternaria radicina* through wounds, rootlets, and uninjured skin

[Inoculations made by means of a spore suspension in carrot decoction in a glass tube sealed on the carrot with vaseline]

Tem- pera- ture, °C.	Dura- tion of experi- ment in days	Inoculated over freshly cut sur- face		Inoculated over uninjured skin		Inoculated over old wound		Inoculated over rootlets	
		Number of carrots used	Number of carrots infected	Number of carrots used	Number of carrots infected	Number of carrots used	Number of carrots infected	Number of carrots used	Number of carrots infected
13	30	5	5	10	4	7	5	7	4
20	28	8	8	17	10	7	5	7	4

METHOD OF INFECTION

It was shown in Table 2 that infection could occur anywhere on the roots. The results recorded in Table 3 indicate not only that *Alternaria radicina* can infect carrot roots through fresh wounds, old wounds, and by way of the remains of small rootlets that are always present on the roots, but that it is able to penetrate the uninjured skin. Infection occurs more slowly through the uninjured skin than by the other methods mentioned, indicating that the skin offers some resistance; in fact only the outer cells (four to five cells deep) were penetrated. The mycelium was found to encircle the cytoplasm in some instances, and in others to ramify through the entire cell. Fresh wounding is shown to be the most predisposing condition to infection.

TABLE 4.—Influence of method of inoculation and types of wounding on infection of carrot roots by *Alternaria radicina*

Container	Inoculated with a spore suspension															Not wounded
	Temperature ° C.	Duration of experiment in days	Method of wounding													
			Crowned			Slice from side of root			Skin scraped from side of root							
			Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected					
Wire basket.....	20	24	62	25	40	41	21	51	70	16	23	49	10	20		
Wire basket.....	22	16	89	39	44	7	2	29	16	23	88	28	28	34		
Moist chamber, cover on.....	22	16	7	4	57	7	2	29	16	23	88	28	28	34		
Moist chamber, cover off.....	22	16	8	6	75	7	2	29	16	23	88	28	28	34		

TABLE 4.—Influence of method of inoculation and types of wounding on infection of carrot roots by *Alternaria radicina*—Continued

Container	Temperature, ° C.	Inoculated with a flake of mycelium and spores												Inoculated by inserting mycelium and spores into tissue of roots				
		Duration of experiment in days	Method of wounding									Inoculated on uninjured skin						
			Crowned			Slice from side of root			Skin scraped from side of root									
			Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected		Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected
Wire basket.....	20	24	53	52	98	---	---	---	---	---	---	---	---	---	---	---	---	---
Wire basket.....	22	16	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Moist chamber, cover on.....	22	16	---	---	---	---	19	19	100	---	---	---	---	7	1	14	19	13
Moist chamber, cover off.....	22	16	---	---	---	---	8	8	100	---	---	---	---	3	0	0	---	68

The results recorded in Table 4 were obtained from experiments designed to determine the influence which the kind of wounding and the method of inoculation have on infection. An examination of the data obtained from the various methods of wounding where the carrots were inoculated by being dipped in a spore suspension reveals considerable variation in the amount of infection. In one instance there was more infection when the carrots were not freshly wounded than when the skin was scraped off. It is believed, however, that these data taken collectively indicate that wounding increases the amount of infection. A partial explanation of the large percentage of infection among the unwounded carrots may be offered on the basis that most of the carrots in storage show some wounding. In fact it is almost impossible to harvest them without some wounding. The unwounded carrots in this instance represent the general run as they occur in storage, no precautions having been observed to eliminate roots with old injuries unless the injury was extensive. An effort was made in some instances to select roots free from injury, but it was next to impossible to do so. It has been observed that *Alternaria radicina* is able to infect carrots through rootlets, and to some extent through the uninjured skin. It is therefore to be expected that the effect of wounding would be merely to increase the chances of infection.

When the carrots were inoculated by placing a small flake of mycelium and spores on the roots, the percentage of infection was higher on all wounded surfaces than when the carrots were inoculated by means of a spore suspension. The percentage of infection on uninjured surfaces was less. Of course the data in these two instances are hardly comparable because the entire carrot, when it was inoculated by means of a spore suspension, was exposed to the inoculum, while in the other case the mycelium and spores were placed only on the uninjured skin.

In the last column of Table 4 are recorded the results obtained when carrots were inoculated by the insertion of a bit of mycelium and spores into the root tissue. This lot was run merely as a check to show that the inoculum was virulent. The percentage of infection is low for this type of wounding and method of inoculation (Table 9).

TABLE 5.—Influence of temperature and wounding on infection of carrot roots by *Alternaria radicina*

Temperature, ° C.	Depression of wet bulb, ° C.	Relative humidity	Duration of experiment in days	Inoculated but not wounded			Wounded and inoculated		
				Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected
20-----	0.9	92	17	18	4	22	12	4	33
18-----	.8	92	17	18	0	0	12	8	66
14-----	.7	91	33	18	1	6	12	8	66
9-----	.9	90	33	18	1	6	12	6	50
9-----	.9	90	33	18	0	0	12	2	17
5-----	.7	88	33	18	0	0	12	0	0
5-----	.5	92	96	17	6	35	12	4	33
Totals-----				125	12		84	32	
Averages-----						10			38

Table 5 contains the results of experiments designed to determine the influence of fresh wounding on infection at different temperatures. The roots were wounded by jabbing a nail into each ten times after the carrots had been inoculated by dipping them in a spore suspension. It will be seen that there is more infection in the wounded carrots at all temperatures except at 5° C., and here the percentage is about the same in the wounded and the unwounded. Temperature seems to have little effect on the number of infections if sufficient time is allowed to permit the maximum to develop at the different temperatures. There is less infection at 20° than at 18° in spite of the fact that 20° is nearer the optimum than 18°. Considering the data collectively, there was 38 per cent infection when the carrots were freshly wounded in contrast to 10 per cent when they were not wounded.

The method of wounding in this experiment was severe, but it shows quite clearly that wounding may become a factor of importance. Just such wounding often occurs in commercial practice when a fork is used in handling the crop.

Another type of wounding occurs during the process of harvesting. Some growers cut off the tops by means of a hoe before the roots are taken from the ground. During this operation many of the crowns are removed and the roots are otherwise injured. This type of wounding is worse than any other, because usually the decay penetrates more deeply when it enters through the crown than through the side of the roots.

TABLE 6.—*Relation of temperature to the growth of Alternaria radicina on carrot agar*

Temperature	Area of colonies	Duration of growing period	Temperature	Area of colonies	Duration of growing period
° C.	Sq. mm.	Days	° C.	Sq. mm.	Days
39.0.....	0	8	5.0.....	0	8
33.6.....	174	8	14.4.....	1,460	17
30.0.....	2,578	8	12.0.....	1,092	17
28.0.....	4,144	8	9.0.....	942	17
24.7.....	3,193	8	5.0.....	71	17
23.0.....	2,801	8	9.4.....	3,486	37
20.0.....	1,831	8	4.4.....	1,361	37
18.4.....	1,261	8	3.0.....	1,279	37
14.4.....	337	8	1.1.....	818	37
12.0.....	258	8	-0.5.....	61	37
9.0.....	87	8			

INFLUENCE OF TEMPERATURE ON THE GROWTH OF THE PATHOGENE IN CULTURE ¹⁰

Three experiments were conducted to determine the range of temperatures at which *Alternaria radicina* will grow on carrot agar and to ascertain the optimum temperature for growth. Table 6 shows the results obtained in these experiments. The results at the temperatures 9.4°, 4.4°, 3°, 1.1°, and -0.5° C. were obtained in one experiment; those at the remaining temperatures are the averages of two experiments. A carrot decoction was made by steaming 500 gm. of peeled and finely chopped carrots for an hour in 1,000 c. c. of distilled water. Twenty gm. of agar was then added to 1,000 c. c. of this decoction. The fungus was grown in 200 c. c. Erlenmeyer flasks plugged with cotton, each flask containing 40 c. c. of the medium. The diameter of the colonies was measured by means of calipers devised for the purpose.¹¹

No growth had occurred at temperatures of 39° and 5° C. at the end of eight days. Thirty-nine degrees is probably near the maximum temperature for growth. Considerable growth occurred at 5° by the end of 17 days. Some growth took place at -0.5° in 37 days. No attempt was made to grow the fungus at temperatures below -0.5°. The amount of growth represented by the area of the colonies increased rapidly as the temperature rose above 5° until it reached 28°, the optimum temperature for growth as indicated by the results obtained at the end of eight days.

A decrease in the amount of growth occurred as the temperature rose above 28°. There was a large increase as the temperature rose from 5° to 14.4° at the end of 17 days and from -0.5° to 9.4° at the end of 37 days. These relations are graphically represented in Figure 2.

¹⁰ The temperature experiments discussed in this paper were conducted in chambers described in an earlier publication. LAURITZEN, J. I., and HARTE, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441-456, illus. 1923.

¹¹ LAURITZEN, J. I. INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEET POTATO IN STORAGE. Jour. Agr. Research 33: 663-676. 1926.

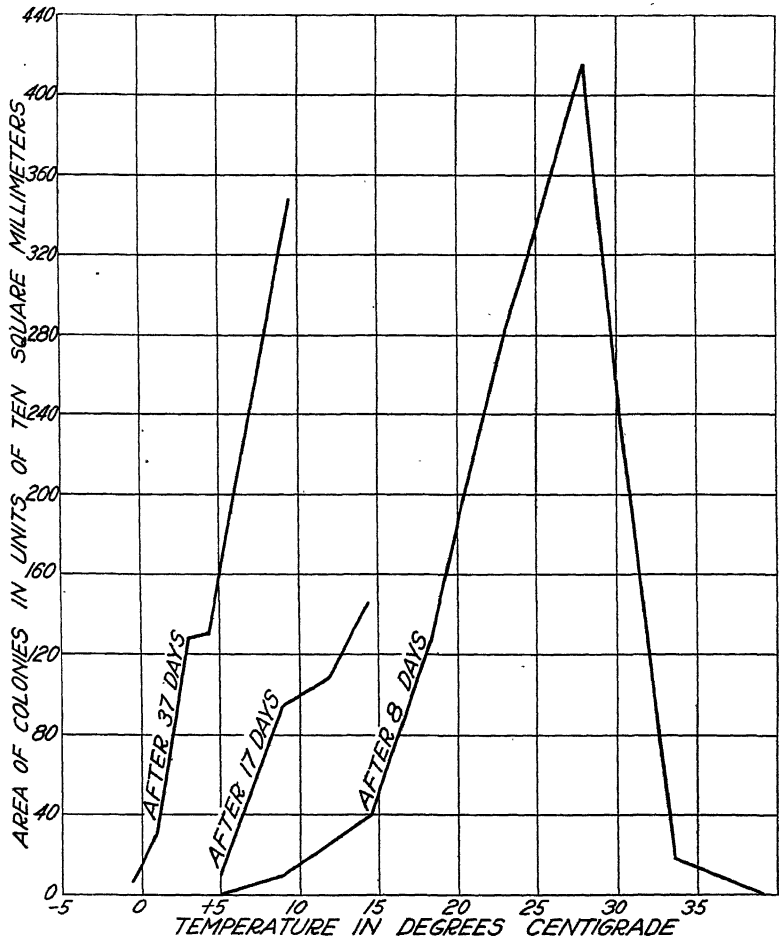


FIG. 2.—Growth of *Alternaria radicina* on carrot agar at different temperatures for 8, 17, and 37 days, respectively

TABLE 7.—Influence of temperature on the development of black rot of carrot after different periods of time

Temperature	Number of lesions measured after 8 days	Average area of lesions	Number of lesions measured after 15 days	Average area of lesions	Number of lesions measured after 21 days	Average area of lesions	Number of lesions measured after 35 days	Average area of lesions
° C.		Sq. mm.		Sq. mm.		Sq. mm.		Sq. mm.
34.0.....	2	17						
32.0.....	39	36	19	53				
28.0.....	53	132	24	504				
24.5.....	55	75	48	313				
23.0.....	52	66	30	247				
20.0.....	57	38	47	117				
19.0.....	56	44	56	155				
14.0.....	46	21	56	49	53	101		
11.0.....			50	47	49	99		
9.7.....	43	8			50	42	53	117
5.0.....	0	0			30	12	49	23
3.5.....					13	16	52	18

INFLUENCE OF TEMPERATURE ON INFECTION AND ON THE DEVELOPMENT OF DECAY

The results recorded in Table 7 were obtained in an experiment that had for its purpose the determination of the influence of temperature and time on the development of black rot.

The carrots were inoculated by inserting a small flake of mycelium and spores into the tissue of the root in two places on opposite sides near the top where the diameter was large. The flakes of mycelium and spores were as nearly as possible of the same size and character in each inoculation. The roots were selected in such a way that those at the different temperatures were comparable in size and shape. Thirty roots were placed at each temperature, and the average area of the lesions was computed from the total number that developed at each temperature. This number was fairly uniform except at the higher temperatures (32° and 34° C.). There was a decrease in the number of lesions at 20° and above with the lapse of time because of decay produced by *Rhizopus* sp. *Penicillium* sp., and *Bacillus carotovorus*. There was also an increased number of lesions with time at the lower temperatures because of the increased number of infections. In all cases except at 34° the number of lesions was sufficiently large to give a representative average.

The optimum temperature for the enlargement of the lesions was found to be 28° C. at the end of 8 and 15 days. As the temperature rose above this point there was a marked decrease in the average size of the lesions. The maximum temperature for infection was not determined, because at temperatures above 30° decay caused by such organisms as *Rhizopus* sp. and *Penicillium* sp. was rapid. Judging by the drop in the size of the lesions at temperatures above 28° the maximum temperature for infection is not much higher than 34°. As the temperature fell below the optimum, the size of the lesions decreased rapidly to a minimum at a temperature between 5° and 9.7° at the end of 8 days, 5° at the end of 21 days, and 3.5° at the end of 35 days. At the end of 15 days no measurements were made below 11°.

The relation of temperature to the enlargement of lesions after different periods of time is shown in Figure 3. There is a sag in the curve made from measurements taken at the end of eight days. This sag tends to straighten out during the successive periods, a fact which seems to be due in part to a proportionately more rapid enlargement of the lesions with the lapse of time at the more favorable temperatures than at the border temperatures. This relation is illustrated in Figure 4. The same relation was found to exist in connection with the study of the influence of temperature and time on the decay of sweet potato by *Rhizopus tritici* Saito;¹² in fact, it was shown more clearly because more comparable intervals of time were selected and the experiments included a greater number of periods.

This behavior may be partially explained on the basis of the differential enlargement of the lesions. Because of the influence of temperature, there is a progressively augmented differentiation of the areas exposed to the action of the fungus with the lapse of time.¹³

¹² LAURITZEN, J. I., and HARTER, L. L. THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS. *Jour. Agr. Research* 30: 793-810, fig. 9, 1925.

¹³ LAURITZEN, J. I. INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEET POTATO IN STORAGE. *Jour. Agr. Research* 33: 663-676, 1926.

Hence not only does the rate of enlargement of the lesions as measured by their areas increase with the lapse of time, but this rate is augmented with the rise in

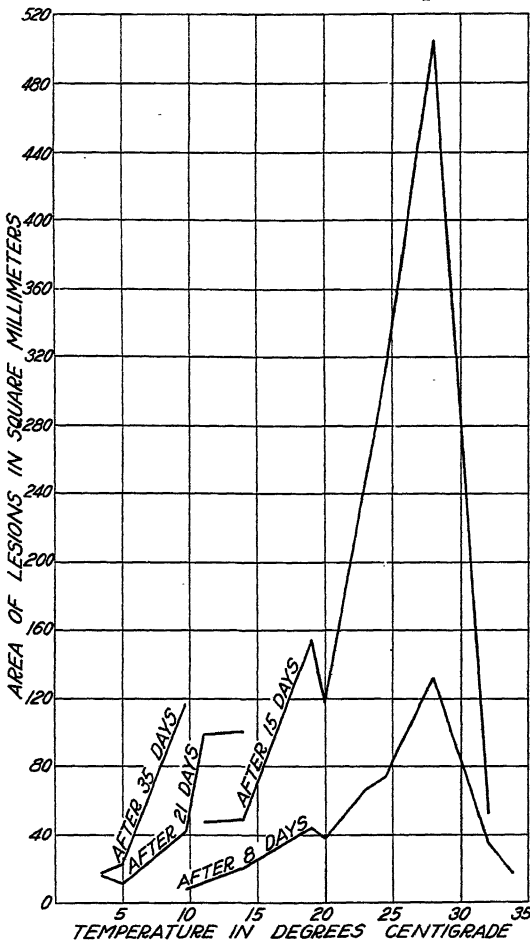


FIG. 3.—Enlargement of lesions with rise in temperature after 8, 15, 21, and 35 days, respectively

with the rise in temperature from the minimum to the optimum. As the temperature rises above the optimum the process is reversed and the rate decreases proportionately with the rise in temperature and the lapse of time.

Infection of carrots by *Alternaria radicina* has occurred at a temperature as low as -0.6°C . (Table 8) in 43 days, but considerably more decay occurred at 0.5°C . in the same time. It will be seen from the results recorded in Table 8 that it is not possible to store carrots at a temperature beyond the range of infection without exposing them to the danger of freezing. All that can be done if the disease is present is to retard infection and the advance of decay by storing the carrots at temperatures ranging from 0° to 2° . This temperature is favorable for the storage of carrots.

TABLE 8.—The lower temperature limits at which *Alternaria radicina* will infect carrots

Temperature, °C.	Duration of experiments, in days	Method of inoculation	Number of carrots used	Number of carrots infected	Percentage of carrots infected	Degree of decay	Check (un-inoculated)	
							Number of carrots used	Number of carrots infected
4.4	62	Dipping carrots in spore suspension.	129	65	50	10 to 100 per cent.		
1.3	158	do.	510	211	41	Lesions mostly small; occasional lesion large.	507	3
.5	43	Inserting mycelium and spores into roots by means of scalpel.	110	98	89	Lesions 1 by 5 mm. to 4 by 8 mm. diameter.		
−.6	43	do.	72	51	71	Infection just started.		

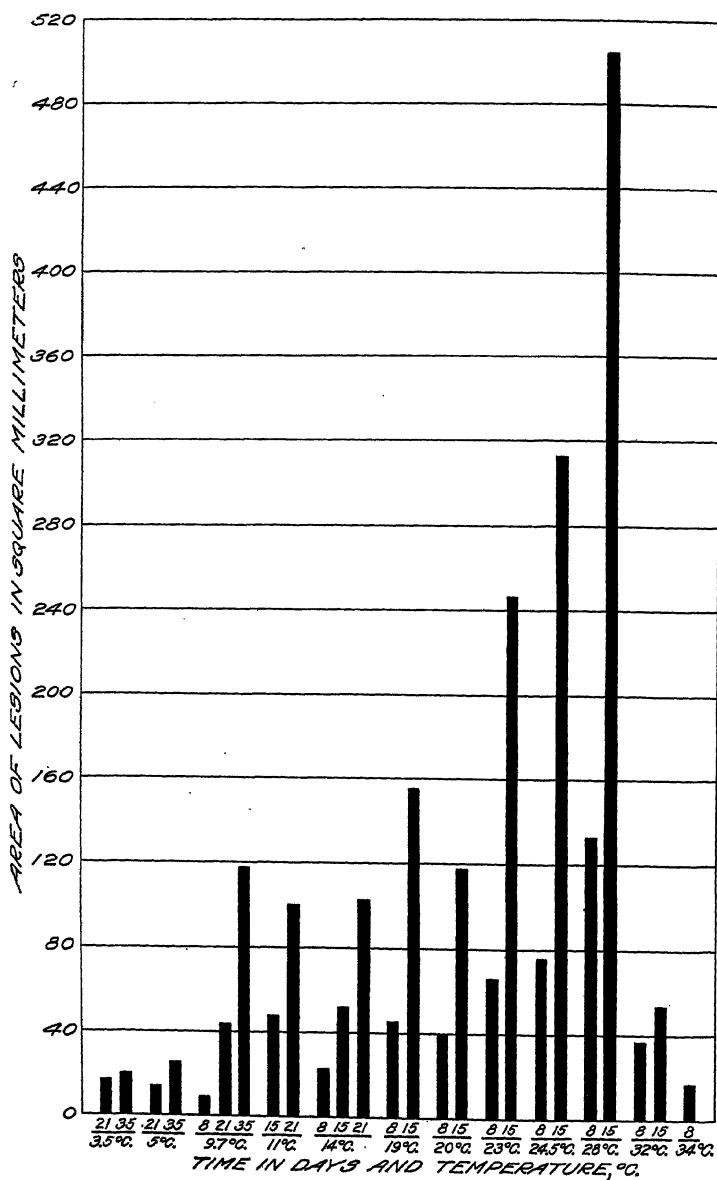


FIG. 4.—Influence of temperature and time on the enlargement of lesions caused by *Alternaria radicina*

VARIETIES AND SUSCEPTIBILITY

A study was made of the susceptibility of 17 varieties of carrots to infection by *Alternaria radicina*. Most of the varieties were used during two seasons.

The seed of the following varieties was obtained from a commercial firm in Paris, France: Jaune obtuse du Doubs, Blanche à collet vert (hors terre), Blanche lisse demi-longue, Rouge demi-longue de Danvers, Rouge longue de Saint-Valéry, Rouge demi-longue de Chantenay, Rouge demi-courte de Guérande, Rouge demi-longue d'Amsterdam, Rouge à forcer Parisienne, and Rouge demi-longue Nantaise. The seed of the following varieties was obtained from a firm in Boston, Mass.: Carter's Nantes, Carter's Early Market, Carter's Red Elephant, Carter's Scarlet Perfection, Carter's Long Forcing, and Carter's Summer Favorite. The seed of Danver's Half Long was procured from a firm in Washington, D. C.

The carrots were grown at the Government experiment farm at Rosslyn, Va., on soil of uniform character and suitable for carrots. The different varieties were planted at the same time and were harvested and stored under the same conditions. Medium-sized roots for the particular variety were selected in each instance. In one experiment the carrots were washed in tap water and allowed to dry under laboratory conditions. A small flake of mycelium and spores was then inserted in the root tissue on one side near the top of the carrot by the use of a small pair of forceps. The quantity of mycelium and spores was as nearly as possible the same in each inoculation. Immediately after treatment the carrots were stored in wire baskets (12 by 12 inches) at a temperature of 13° C. in a room provided with air exchange.

TABLE 9.—Susceptibility of 12 varieties of carrot to attack by *Alternaria radicina* when roots were inoculated by insertion of mycelium and spores

Variety	Number of carrots inoculated	Number of carrots infected	Percentage of carrots infected	Average diameter of lesions in millimeters
Rouge demi-longue de Danvers	39	39	100	6.9
Carter's Nantes	39	35	97	7.1
Rouge longue de Saint-Valéry	39	36	92	7.0
Rouge demi-longue Parisienne	40	40	100	7.9
Rouge demi-courte de Guérande	40	40	100	4.6
Rouge demi-longue de Chantenay	40	38	95	5.7
Carter's Long Forcing	38	37	97	6.5
Carter's Scarlet Perfection	41	40	97	6.3
Rouge demi-longue Nantaise	37	37	100	8.1
Carter's Early Market	40	40	100	8.9
Rouge demi-longue d'Amsterdam	40	35	88	7.0
Carter's Red Elephant	41	41	100	9.6

The results recorded in Table 9 were obtained after 11 days. There was very little difference either in the percentage of infection or the average diameter of the lesions in the 12 varieties used. Rouge demi-longue d'Amsterdam showed the lowest percentage of infection but not the smallest average diameter of the lesions. This method provides a severe test of pathogenicity, first because it eliminates any resistance that may be offered by the skin of the roots, and, second, because it provides for a large quantity of inoculum at the

point of inoculation. An effort was therefore made to determine the susceptibility of a number of varieties under conditions of infection that simulate more nearly the conditions that obtain in storage (except where infection occurs through the tops).

Table 10 contains the results of experiments conducted during two seasons. In each group of experiments the carrots were carefully washed to avoid wounding, dried, and inoculated by dipping in a spore suspension of the pathogene. In 1924 they were stored at a temperature of 13° C. and a relative humidity ranging from 75 to 90 per cent; in 1925 they were stored at a temperature of 10° and a relative humidity ranging from 88 to 96 per cent.

TABLE 10.—*Susceptibility of 17 varieties of carrot to attack by Alternaria radicina when the roots were inoculated by dipping in a spore suspension*

Variety	1924			1925			Total percentage of carrots infected
	Number of carrots used	Number of carrots infected	Percentage of carrots infected	Number of carrots used	Number of carrots infected	Percentage of carrots infected	
Blanche à collet vert (hors terre).....				24	1	4.2	4.2
Blanche lisse demi-longue.....				18	2	11.1	11.1
Carter's Early Market.....	43	5	11.6	44	3	6.8	9.2
Carter's Long Forcing.....	47	3	6.4	117	0	0	1.3
Carter's Nantes.....	45	0	0	70	5	7.1	4.3
Carter's Red Elephant.....	43	3	7.0				7.0
Carter's Scarlet Perfection.....	47	6	12.8			5.0	9.2
Carter's Summer Favorite.....				42	10	23.8	23.8
Danver's Half Long.....	44	1	2.3	46	1	2.2	2.2
Rouge demi-longue de Danvers.....	44	3	6.8	26	2	7.7	7.1
Jaune obtuse du Doubs.....				25	1	4.0	4.0
Rouge à forcer Parisienne.....	45	15	33.3	45	11	24.4	28.9
Rouge demi-courte de Guérande.....	44	2	4.5	35	0	0	2.5
Rouge demi-longue d'Amsterdam.....	43	1	2.3	54	2	3.7	3.1
Rouge demi-longue de Chantenay.....	46	3	6.5	34	5	14.7	10.0
Rouge demi-longue Nantaise.....				89	6	6.7	6.7
Rouge longue de Saint Valéry.....	45	0	0				0

All varieties proved to be susceptible except Rouge longue de Saint Valéry, which, however, was used in only one year. In the case of three other varieties there was no infection in one or the other of the years, and it is therefore possible that this variety might also have become infected if it had been used in another trial. It was shown (Table 9) that *Alternaria radicina* is capable of infecting this variety when it is inoculated by inserting mycelium and spores into the tissue, and the quantity of decay was as great as in most of the other varieties.

There is some variation in the percentage of roots infected, but for the most part these differences lie within the limits of experimental error.

There are two outstanding cases of susceptibility, Rouge à forcer Parisienne and Carters' Summer Favorite. In the case of the former the percentage of infection was large both in 1924 and 1925. There is a possible explanation for susceptibility in this instance. Rouge à forcer Parisienne is a short stubby carrot which grows very rapidly. In consequence, abundant growth cracks develop in the roots, and although most of these heal over the healing is imperfect, leaving areas unprotected by the skin tissue. It is true that Rouge demi-courte de Guérande is also nearly always growth-cracked and does

not show a marked susceptibility to infection, but the cracking seems to heal more perfectly.

Carter's Summer Favorite, judging by one trial only, seems particularly susceptible. More work will be done on this variety.

It is important to note that Danver's Half Long, America's greatest commercial variety, shows a consistently low percentage of infection. Rouge demi-longue de Danvers, which is supposed to be the same variety, shows consistently a higher percentage of infection. Whether or not we are dealing here with different strains, is not known. It is known, however, that there is considerable variation¹⁴ in Danver's Half Long in the size and shape of the roots and that the roots from French seed have been consistently larger than those from American seed. In any case Danver's Half Long, whether of American or French origin, ranks with the less susceptible varieties.

DISCUSSION AND SUMMARY

Carrot roots free from black rot and from contamination with the pathogene do not become infected when stored in a house which has not been contaminated by the previous storage of black-rot carrots. It would seem, therefore, that the disease has its origin in the field.

Carrot foliage inoculated with a spore suspension of *Alternaria radicina* became infected with a disease which very closely resembles carrot blight. Roots from plants whose foliage was inoculated showed no evidence of infection at harvest time (early November), but showed heavy infection at the termination of the storage period (April and May). These results point to a possible connection between foliage infection and root infection, but the negative results obtained from attempts to find the foliage disease under field conditions tend to discredit this view.

A larger number of infections from foliage inoculation occur at the sides of roots than at the crown, showing that it is not necessary for the pathogene to pass directly through the foliage to the roots. It would seem that the infections on the sides of the roots at least were produced by contamination with the pathogene from one of the following sources: (1) From the washing of spores from the foliage to the soil and roots; (2) from contact with foliage at harvest time, and (3) from the soil before and at the time of digging. Since *Alternaria radicina* is a vigorous saprophyte and can live indefinitely on dead organic matter, it would appear reasonable to believe that it might live in the soil, especially if decaying carrot roots and foliage were present. The roots could thus become contaminated through the soil and in turn infected during storage. This conception would account for the black rot of the roots in storage in the absence of the foliage disease.

Alternaria radicina is capable of infecting carrot roots through the uninjured skin. Such infection occurs very slowly, penetrating only a few cells deep. The mycelium sometimes surrounds the cytoplasm and at other times ramifies throughout the entire cell.

It is not necessary for the fungus to gain entrance through the uninjured skin; it can enter readily through old wounds, rootlets, and through fresh wounds. Old wounds are abundant in all stored

¹⁴VILMORIN-ANDRIEUX ET COMPAGNIE. THE VEGETABLE GARDEN; ILLUSTRATIONS, DESCRIPTIONS, AND CULTURE OF THE GARDEN VEGETABLES OF COLD AND TEMPERATE CLIMATES. English ed. pub. under the direction of W. Robinson. Ed. 3. p. 197-198, illus. London. 1920.

carrots, for it is impossible to harvest and store them without some wounding. Fresh wounding increases the opportunity for infection, the worse it is the greater the infection.

Temperature has little effect on the number of infections if sufficient time is allowed to permit of the maximum infection, except at the border temperatures.

Alternaria radicina will grow on carrot agar at temperatures ranging from -0.5° to 33.6° C. There was no growth at 39° in 8 days, but there was considerable growth at -0.5° in 37 days. Growth might have occurred at temperatures between 33.6° and 39° or below -0.5° had these temperatures been employed.

The optimum temperature was found to be 28° C. The decrease in the rate of growth as represented by the area of the colonies was rapid as the temperature rose above or fell below this point.

The temperature range for infection of carrots by *Alternaria radicina* extends from -0.6° to 34° C. This range is slightly wider than that for growth of the fungus, but this fact can be explained on the basis of the temperatures employed in the two instances. It is impracticable to determine the upper temperature limit of infection because carrots decay rapidly at the higher temperatures from the attack of such fungi as *Rhizopus* sp. and *Penicillium* sp. It is possible that infection might have been obtained at temperatures below -0.6° had these been employed, but a temperature even as low as -0.6° is too low for the practical storage of carrots. The best that can be done if the black-rot disease is present is to store the carrots at temperatures of from 0° to 2° C. and thus reduce the amount of infection and decay to a minimum.

The optimum temperature for infection and decay of carrots by *Alternaria radicina* is about 28° C. as shown by the results both after 8 and after 15 days. This optimum is identical with the optimum for growth of the pathogene. It is not thought, however, that the optima are as definite as these data would indicate. It is expected that they would vary somewhat if slightly different temperatures were employed.

There is a rapid decrease in the rate of enlargement of the lesions as the temperature is raised above or lowered below 28° C., particularly when it is raised above. There is a lag in the rate of enlargement of the lesions and in the number of infections in the early stages of infection and decay at temperatures below 19° , but the tendency is for this lag to be recovered with time.

The rate of growth of the fungus as represented by the area of the colonies in carrot agar is much greater at all temperatures than the rate of enlargement of the area of lesions. This fact is well illustrated in Figures 3 and 4 by the curves drawn from data obtained at the end of eight days.

Alternaria radicina has been shown to be capable of infecting 17 varieties of carrots. There is little difference in the number of infections or degree of decay as measured by the increase in the diameter of the lesions in 12 varieties when the roots are inoculated by inserting into them a small flake of mycelium and spores and storing them under the same conditions.

When carrots were inoculated by being dipped in a spore suspension, the Carter's Summer Favorite variety showed a marked susceptibility in the one experiment in which it is used. Rouge à forcer Parisienne

was the most pronouncedly susceptible variety. The susceptibility of this variety was consistently high during the two seasons in which it was used. Danver's Half Long, whether the seed was obtained in the United States or from France, was one of the least susceptible of the varieties. When the roots of Rouge longue de Saint Valery were dipped in a spore suspension this variety showed no infection in the one trial in which it was used. This fact does not prove its immunity. Three other varieties showed an absence of infection in one experiment and the presence of infection in another. Rouge longue de Saint Valery did become infected when inoculated by inserting a flake of mycelium in the roots.

THE RELATION OF SEASONAL FACTORS TO QUALITY IN SWEET CORN¹

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INTRODUCTION

In the course of studies designed to throw light upon the factors concerned with the quality of sweet corn grown for canning purposes, the results of which have been reported in part in an earlier paper (3),² the degree of maturity of the corn at the time of harvesting was found to be of prime importance. Since, however, the rate of coming to maturity is known to be affected by seasonal conditions, and since in the earlier experiments corn was studied from plantings made in the early spring only, it seemed desirable to determine to what extent quality in sweet corn is affected by the season during which it comes to canning maturity.

Accordingly, in the spring of 1924 plans were laid for a series of plantings to be made at intervals throughout the spring and summer, and both field and laboratory studies to be made of the corn derived from these plots. In the present paper the details of these experiments will be set forth and the results of the work discussed.

PLAN OF WORK

Two varieties—Golden Bantam, representing the early corns, and Stowell's Evergreen, representing the late—were selected for the experiments.

The ground chosen for the experimental plots was located at Arlington Experiment Farm, on the Virginia side of the Potomac River near Washington, in that portion known as the "flats," which was formed some years ago by hydraulic fill from the river channel. In contour the land was nearly level. The soil was a deep, rich loam provided with tile subsurface drainage.

Beginning at the earliest feasible date, it was planned to make plantings of each variety at intervals of 10 days throughout the season, or for a period sufficiently long to be sure that the entire growing season would be utilized. Unfavorable seasonal conditions and other factors interfered to some extent with the working out of this plan, but in all 13 plantings were made of Golden Bantam and 12 plantings of Stowell's Evergreen. The actual planting dates were: April 28, May 5, 10, 23, and 31, June 7, 17, and 27, July 9, 19, and 28, and August 7, and 19.

The seed was well selected and was purchased in sufficient quantity to make possible the seeding of all plots from the same lots of seed.

The seed was drilled in by hand machine and the seeding made purposely heavy so as to assure a good stand. When the plants were

¹ Received for publication June 19, 1926; issued December, 1926.

² Reference is made by number (italic) to "Literature cited," p. 1072.

sufficiently large each plot was thinned, leaving vigorous plants spaced at about 9 inches in the row in the case of Golden Bantam and from 14 to 16 inches in the case of Stowell's Evergreen.

Standard cultural practices were used throughout and all plots received like treatment.

Field records were kept of the dates when tassels were first observed in the various plots and of the time and rate at which the silking occurred, tags of different colors marked with the proper dates being attached daily to the plants showing new silks, as in previous experiments.

Considering the day on which the silks of individual ears first appeared as the starting point from which to calculate the age of the corn, samples were taken when the corn was five days old and at intervals of five days thereafter throughout the entire period of development. Upon these samples determinations were made of the weight of the whole ear, husk, cob, and cut corn, and from each lot at these various stages samples of the cut corn were taken for chemical analysis. Beginning with corn at the 10-day stage, tests were made at 5-day intervals thereafter to determine the degree of toughness of the kernels as measured by their resistance to puncture; and, beginning at the 15-day stage, practical canning tests were performed at 5-day intervals on corn from each of the plantings until it was well past canning maturity. In this way it was possible to follow through the entire history of each plot of corn; to study the relation of seasonal conditions to the vegetative activities of the plant; to observe the rate at which maturity progressed; to ascertain the nature and degree of chemical transformations occurring within the grain; and, finally, to determine the effect of these various factors on the quality of the canned product.

SEASONAL DATA

The meteorological data for the period April 16 to November 30, inclusive, are given in some detail in Figure 1.

This chart is self-explanatory except in two particulars—the curve for mean daily temperature is based upon hourly readings throughout the entire period, the combined records showing the actual temperature conditions under which the corn was grown and studied; the other exception is in respect to the sunshine and cloudiness records. The height of the daily columns is here determined by the number of hours of possible sunshine, the variable width of the band formed by these daily columns corresponding to the varying day lengths as the season advanced.

Particular attention is called to the curve showing the daily mean temperature as compared with the normal mean for the same period. It is noted that during the early spring, that is, during the latter half of April and the first week of May, the temperature did not vary much from normal. The latter two-thirds of May and the first half of June were abnormally cool, the maximum temperature frequently falling some distance below the normal mean for that period. From the middle of June to the first of September the average temperature did not vary much from normal, though for a few days in the middle of August it remained somewhat below normal, and the month closed with a short hot period which reached its highest temperature

on the 31st. With the beginning of September there was an abrupt change, the temperature dropping considerably below normal and remaining abnormally cool for almost the entire month. October showed the usual fluctuations. Several light frosts were recorded during the month, but the first killing frost occurred on the night of the 21st. The first half of November was unusually warm, with occasional sharp drops in temperature. On the night of the 16th there was a hard freeze, and for several days the temperature was considerably below normal. For the last third of the month about average temperature conditions prevailed.

The season was a particularly favorable one for observing the effect of variations in rainfall on the development and maturing of sweet corn, as during this period everything from flood to prolonged drought was encountered. Immediately after the third planting was made heavy rains up the valley of the Potomac forced the river out of its bounds, and for one or two days those plantings which had been made, as well as the adjacent land where later plantings were to be made, were under water. The remainder of May was abnormally wet. The rainfall for June was a little below normal but was fairly well distributed. With the exception of one abundant rain about the 8th of the month, July was practically rainless,

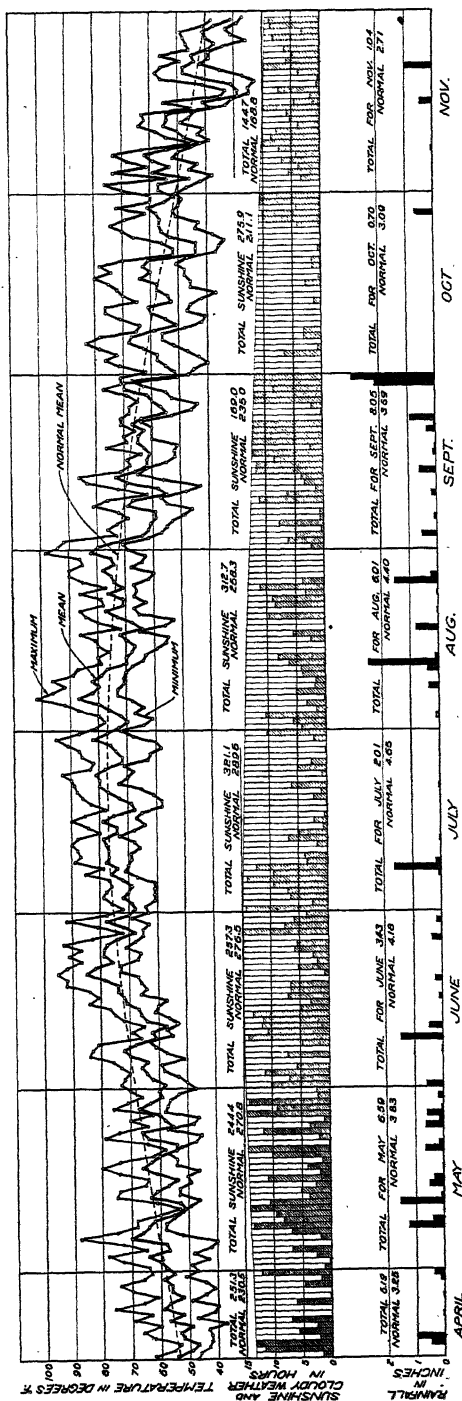


Fig. 1.—Meteorological data for the season of 1924 at Arlington Experiment Farm, Rosslyn, Va.

the total rainfall for the month being less than one-half the normal quantity; and it was not until about the 12th of August that sufficient rain fell to be of material benefit to the crop. With the breaking of the drought, the remainder of August had about the normal amount of rain. The precipitation during September was well distributed and about normal for the month, up to the last two days, when there was a downpour totaling somewhat over 5 inches. October was entirely rainless except for the 28th, when 0.7 inch of rain was recorded. November received about 60 per cent of its normal precipitation.

Brief notice should be taken of the sunshine record for this period. During May and June there was a small deficiency in sunshine. July, which normally has a slightly higher percentage of sunshine than any other month, during this season had an excess of a little over 31 hours. August had a considerable excess, amounting to over 54 hours, whereas September had a very considerable deficiency. The amount of sunshine for October was far above normal, concomitant with the rainless period. The data for November show a deficiency.

The importance of these various seasonal factors as affecting sweet corn will be shown as the results of this study are set forth.

TIME AND RATE OF SILKING

The time of planting has a very important bearing on the development of the sweet-corn crop. It has been a matter of common observation that very early planting does not necessarily result in a particularly early crop, and even in those regions where the growing season is short and frost hazards are encountered in the early fall the grower has some leeway as to the time when the planting may be done and still have the corn come to canning maturity. In other words, experience has shown that a difference of several days to a week in the time of planting in the early spring makes no noticeable difference in the time at which the corn comes to harvest.³

In the present experiments an opportunity was offered to study this matter rather closely, and the results of these observations are illustrated in Figures 2 and 3. No graphs are included in these figures for the last planting of each variety, since these plantings had not reached the silking stage when the crop was killed by frost. The individual graphs are so placed as to show by the ordinates the relative positions of the different plantings with respect to planting dates, and by the abscissas the period of time during which silking occurred. The height of the columns for different days in the individual graphs shows the percentage of the total silks that made their appearance on individual days.

In a chart constructed in this way the graphs for the different plantings, provided no seasonal or other factors modified the rate at which crop growth proceeded, would show the peak of silking at a definite interval from the planting date, and a line drawn through the points on the chart representing these dates would be a straight line. Such a line is represented in Figures 2 and 3. The position of this line with respect to the graphs in each of these figures was determined by taking as the interval between planting and silking the shortest

³In this connection, reference is made to the experimental work on field corns by Morrow and Hunt (8), Foster (4), Morrow and Bone (9), and Alberts (1).

period actually observed in the tests. In the case of Golden Bantam this period was 55 days, and in the Stowell's Evergreen, 63 days.

In the case of Golden Bantam it is seen that the first planting required about 25 days longer to arrive at the peak of silking than did the seventh and eighth. The second and third plots which were planted five days apart arrived at the peak of silking on the same day, and required 22 days more than the seventh and eighth plots to arrive at the same stage of development. In later plantings the interval from planting to silking was gradually reduced until the shortest was reached in the seventh and eighth plots. In the succeeding plantings there was a gradual lengthening of the period again, which was particularly marked in the last planting.

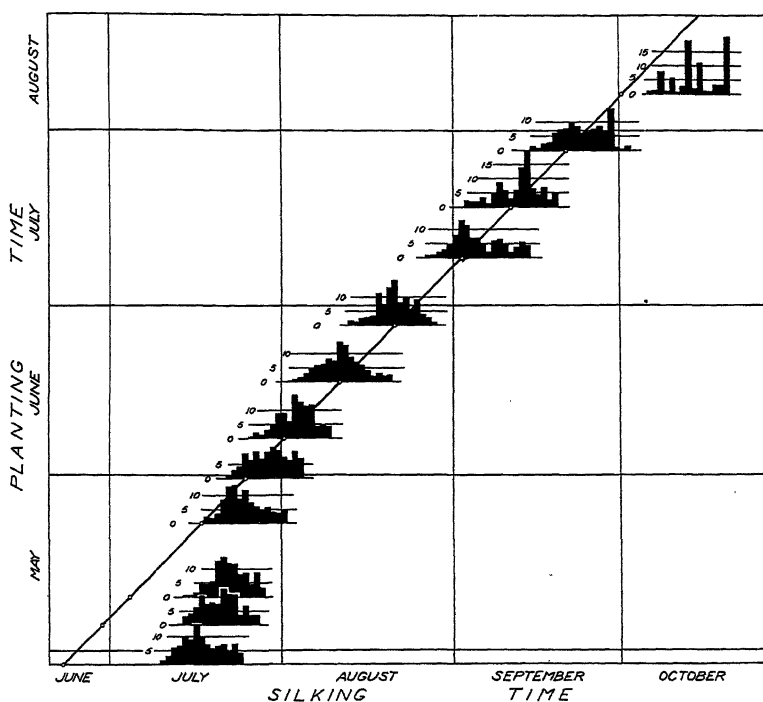


FIG. 2.—Effects of seasonal conditions on the time and rate at which the different plots of Golden Bantam sweet corn came into silking

In the case of Stowell's Evergreen the picture is entirely similar, though the variations from the minimum period are even more pronounced than has been observed in the case of Golden Bantam. Here the interval between planting and midsilking, in the case of the first planting, was about 30 days longer than that required by the seventh and eighth plantings to reach a comparable stage of development, and a very considerable lengthening of the period was observed in the last planting.

The experimental findings, therefore, accord with practical observations in showing that very little is to be gained with respect to earliness of the sweet-corn crop by very early planting, and that the grower desiring to have a succession of harvests at more or less defi-

nite intervals will need to time his early spring plantings at considerably longer intervals than with the late spring plantings.

The factors responsible for the variations here observed and the further significance of these variations as affecting the harvest will be discussed at greater length further on in this paper.

VEGETATIVE CONDITION OF CROP

The effect of seasonal conditions upon the development of the different plots of corn was very striking. Judged purely by appearance, one would have considered the various plantings, particularly of Stowell's Evergreen, as made up of a number of different varieties

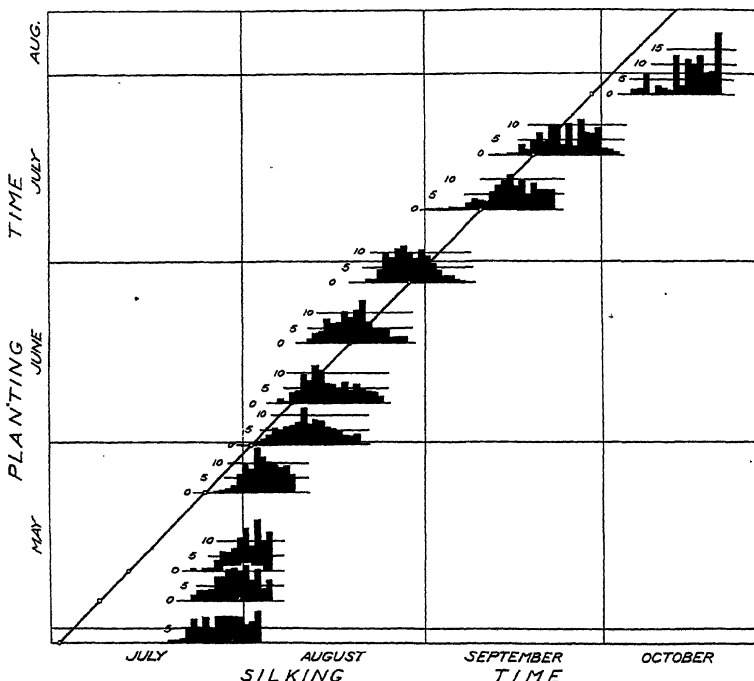


FIG. 3.—Effects of seasonal conditions on the time and rate at which the different plots of Stowell's Evergreen sweet corn came into silking

In those plots whose normal vegetative growth period fell within the season of drought the plants were stunted, and in those cases in which silking occurred during this period the ears were particularly affected. Often ears that from external appearance were judged to be normal on husking would exhibit large full-grown cobs with only scattering kernels or almost none at all. In these cases difficulty was encountered in getting sufficient corn to meet sampling needs. From the standpoint of yield, such corn was practically a total failure.

Figure 4 gives some idea of the effect of seasonal conditions on the vigor of the plants in the different plots of Stowell's Evergreen. The data are based upon the averages of numerous measurements made upon the mature stalks in the different plots, with the excep-

tion of the last two in which a killing frost prevented the development of the plants to full vegetative maturity.

Attention is called particularly to the height of the corn and the position of the ears in plots 4, 5, and 6 as indicated in the chart. The corn in these plots passed a considerable portion of their actively vegetative periods during the season of drought recorded as between the middle of July and the 12th of August.

It is seen from this and the preceding figures that the quantity and distribution of the rainfall, as well as the temperature conditions prevailing during the growing period, have a very important bearing on the vegetative development of the sweet-corn plant.

YIELDS

No attempt was made in the present study to determine the effect of seasonal conditions on total yields of corn from the various plots, the conditions under which the experiments were conducted not making it feasible to secure such records. In general, the vegetative condition of a field of corn is a fairly good index of yields, and reference to Figure 4 will give some notion of relative yields from the different plots in the present case. It should be borne in mind, however, that in point of time the development of the ear lags somewhat behind the

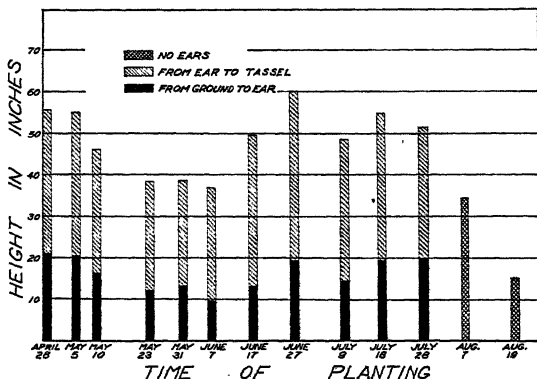


FIG. 4.—Effect of seasonal conditions on the vegetative development of the different plots of Stowell's Evergreen sweet corn. The heavy black portions of the individual graphs give the distance from the ground to the base of the ear, and the crosshatched portion the distance from the ear node to the base of the tassel.

vertical growth of the corn plant, and factors affecting the physical condition of the corn plant would be reflected in the ear at a slightly later time than in the stalk. Field observations showed that the yield from the third plot was relatively less than the graph for that planting would indicate and somewhat more than would be inferred from the graph for the sixth plot.

As in previous studies (3), records were kept of the rate of development of the ears and the proportion of husk, cob, and cut corn in the ears at different stages of maturity. The data secured are presented in Table 1. Too close analysis of the figures in this table should not be made, as the number of ears available for individual tests were too few for accurate determination of averages. Irregularities in the series of figures indicating the progress of development of the different elements of the ear are occasionally observed in the table, which are due in part to this element of error and in part to the effect of drought on some of the corn, which affected the percentage yields. The full effect of drought, however, is not shown, since abnormal ears, as far as possible, were excluded from the tests.

TABLE 1.—Rate of development of the sweet-corn ear and the changes occurring in the proportion of husk, cob, and cut corn during growth of Golden Bantam and Stowell's Evergreen varieties, planted at intervals throughout the season of 1924 at Arlington Experiment Farm, Rosslyn, Va.

Plant- ing	Date of planting, 1924	Golden Bantam							Stowell's Evergreen						
		Date of sampling days	Num- ber of ears taken	Aver- age weight husked ears	Aver- age weight of ears per ear	Per cent of husk	Per cent of cob	Per cent of cut corn	Date of sampling days	Num- ber of ears taken	Aver- age weight husked ears	Aver- age weight of ears per ear	Per cent of husk	Per cent of cob	Per cent of cut corn
1	Apr. 28	July 16	20	Grams 23.0	Grams 129.8	68.7	15.8	15.5	July 30	22	Grams 128.4	Grams 50.4	60.8	23.8	15.4
		July 22	15	21.5	120.1	46.6	26.5	13.6	Aug. 5	10	138.7	19.8	43.2	44.0	12.8
		July 29	13	68.3	164.6	40.9	35.5	23.6	Aug. 7	11	257.7	33.1	35.6	35.6	27.8
		Aug. 4	10	97.3	182.0	27.0	29.1	43.3	Aug. 15	7	107.9	73.5	30.5	26.2	43.3
		Aug. 14	25	133.0	80.0	30.8	25.6	43.3	Aug. 23	6	235.2	173.2	31.7	26.2	46.1
2	May 5	Aug. 16	12	109.5	170.6	23.2	23.2	53.6	Aug. 30	5	262.1	211.0	26.2	20.8	53.0
		July 21	5	23.3	75.4	69.2	17.8	13.0	Aug. 31	5	398.0	54.0	64.5	23.3	19.2
		July 28	10	59.5	105.2	43.5	36.2	20.3	Aug. 4	10	18.6	18.6	38.2	41.5	20.3
		Aug. 1	15	81.3	127.6	36.4	40.6	23.0	Aug. 8	15	133.5	43.9	37.6	38.0	24.3
		Aug. 11	20	144.1	221.6	34.9	25.2	39.9	Aug. 18	20	165.0	64.5	34.8	28.1	37.1
3	May 10	Aug. 11	5	118.0	78.0	29.4	23.9	46.7	Aug. 26	6	228.3	130.0	28.6	24.8	46.6
		Aug. 22	30	29.5	92.2	68.1	17.5	14.4	Sept. 3	6	297.5	194.1	27.5	19.8	52.7
		July 23	5	29.5	92.2	68.1	17.5	14.4	Aug. 1	5	262.5	190.8	62.7	25.4	11.9
		July 30	10	57.0	116.0	50.6	21.2	18.1	Aug. 15	10	55.0	17.5	55.6	25.4	11.9
		Aug. 1	15	82.0	142.2	38.4	26.4	18.1	Aug. 15	10	100.8	23.7	55.6	34.0	10.4
4	May 23	Aug. 8	11	113.1	174.5	33.2	25.0	35.6	Aug. 15	7	161.0	70.5	40.7	33.3	26.0
		Aug. 21	25	111.2	171.2	30.5	25.0	44.5	Aug. 22	7	244.2	143.5	36.7	26.1	37.2
		Aug. 23	6	196.6	136.6	30.5	19.5	50.0	Aug. 26	6	260.0	200.0	23.6	23.6	46.6
		July 23	5	25.7	72.5	64.6	10.7	15.7	Sept. 3	6	273.1	151.6	25.6	23.5	54.1
		July 30	10	56.3	110.8	46.5	33.0	15.7	Aug. 4	5	55.0	17.5	62.7	25.4	11.9
5	May 31	Aug. 5	15	129.2	152.9	36.8	26.9	20.4	Aug. 8	10	182.4	30.0	49.4	37.6	13.0
		Aug. 8	10	157.0	104.5	33.5	28.3	26.9	Aug. 15	15	230.4	78.0	36.5	38.1	25.4
		Aug. 22	25	123.3	181.6	32.2	25.2	42.6	Aug. 22	7	195.0	219.3	36.4	26.7	36.9
		Aug. 25	6	176.6	143.3	18.9	21.2	59.9	Sept. 2	5	208.3	145.0	30.4	27.2	42.4
		July 28	5	20.0	66.3	69.9	16.7	13.4	Sept. 4	5	266.0	183.0	27.0	22.6	50.2
		Aug. 3	10	53.5	108.2	50.6	32.4	17.0	Aug. 8	10	115.5	44.5	61.5	24.0	14.5
		Aug. 10	15	174.5	148.5	41.8	30.7	27.5	Aug. 14	10	189.2	101.7	46.4	36.9	16.7
		Aug. 20	20	168.9	188.9	37.5	24.0	36.5	Aug. 21	15	270.0	119.0	38.3	44.0	17.7
		Aug. 23	4	82.8	127.3	26.3	20.0	49.6	Aug. 28	7	338.6	230.5	35.8	30.8	33.4
		Aug. 25	9	134.4	97.2	24.0	21.0	53.0	Sept. 4	6	246.4	109.3	28.4	22.4	40.2
		Aug. 27	9	176.6	134.4	24.0	21.0	53.0	Sept. 11	6	439.1	323.3	26.5	22.3	51.2

6	June 7	Aug. 1	5	14	76.8	18.9	8.0	74.5	15.0	10.5	Aug. 12	5	13	140.0	57.2	20.0	50.2	26.6	14.2
		Aug. 8	8	11	119.5	55.4	14.0	53.7	34.6	11.7	Aug. 20	10	14	327.1	121.7	32.1	46.5	36.4	14.2
		Aug. 15	15	10	163.0	88.0	32.0	46.1	34.3	19.6	Aug. 26	15	7	337.1	205.0	175.0	46.5	36.4	14.2
		Aug. 22	22	7	170.7	108.5	82.1	36.5	15.4	48.1	Sept. 2	20	7	357.8	246.0	143.7	31.0	22.5	53.1
		Aug. 29	29	6	167.5	122.5	85.0	27.0	22.3	50.7	Sept. 9	25	6	361.6	266.6	208.3	34.4	22.5	39.7
7	June 17	Sept. 5	30	6	167.5	122.5	85.0	27.0	22.3	50.7	Sept. 15	30	7	364.2	188.5	105.1	28.7	31.6	39.7
		Aug. 21	5	18	72.2	23.6	8.6	67.4	20.7	11.9	Aug. 18	5	14	136.4	46.7	17.1	65.8	21.7	12.5
		Aug. 14	10	12	117.0	51.6	20.3	56.0	26.6	16.4	Aug. 23	15	13	275.9	150.4	53.3	44.0	35.6	19.5
		Aug. 22	15	9	164.4	106.6	31.1	37.3	33.7	18.4	Aug. 29	20	7	323.5	195.6	90.0	30.9	32.6	27.8
		Aug. 29	26	7	186.4	114.3	36.5	30.2	31.0	30.2	Sept. 8	20	6	443.3	200.1	177.5	32.9	27.4	40.0
8	June 27	Sept. 6	25	7	202.8	137.4	110.0	38.8	20.4	31.8	Sept. 11	25	6	415.0	333.3	231.6	19.7	24.5	55.8
		Sept. 13	30	5	214.0	167.0	111.0	26.8	21.4	31.8	Sept. 19	30	6	470.0	342.5	245.8	27.2	20.5	52.3
		Aug. 18	5	14	62.1	15.7	6.8	74.5	14.3	10.9	Aug. 28	5	11	170.9	74.0	26.8	56.7	27.6	15.7
		Aug. 25	10	16	126.6	47.5	17.8	48.5	24.0	26.9	Sept. 4	10	11	294.5	164.0	54.5	44.4	37.1	18.5
		Sept. 2	15	7	151.4	88.5	42.9	41.6	30.1	28.3	Sept. 10	15	7	391.4	226.2	89.3	41.5	35.7	22.8
9	July 9	Sept. 9	20	8	194.3	122.5	68.7	37.1	27.6	35.3	Sept. 16	20	6	428.3	208.3	140.8	37.5	31.1	32.8
		Sept. 15	25	9	180.0	115.0	72.2	36.2	23.7	40.1	Sept. 22	25	7	472.8	307.1	160.0	35.1	30.1	33.8
		Sept. 22	30	8	201.2	138.7	95.6	31.1	21.4	47.5	Sept. 27	30	6	508.3	375.0	221.6	26.3	30.1	43.6
		Sept. 29	35	8	145.0	90.0	56.2	38.1	23.2	38.7	Sept. 27	35	a 3	453.3	321.6	235.0	20.1	19.1	51.8
		Oct. 14	35	8	145.0	90.0	56.2	38.1	23.2	38.7	Oct. 17	40	6	268.3	204.1	130.8	24.0	27.3	48.7
10	July 18	Sept. 9	5	14	73.9	18.2	8.2	75.4	13.5	11.1	Sept. 22	5	15	132.6	26.3	8.3	80.3	13.5	6.2
		Sept. 15	10	14	115.0	30.0	10.7	74.0	16.7	9.3	Sept. 29	10	8	225.0	101.8	32.5	54.8	32.5	12.7
		Oct. 2	15	10	142.0	54.0	18.0	62.0	25.4	12.6	Oct. 10	15	12	245.0	122.5	30.8	53.1	31.1	12.7
		Oct. 9	20	6	157.0	67.0	24.0	57.5	27.3	13.2	Oct. 16	20	12	271.9	136.8	56.6	44.8	41.3	13.9
		Oct. 16	25	6	211.6	105.0	44.1	50.5	28.7	20.8	Oct. 23	25	7	271.9	136.8	56.6	44.8	41.3	13.9
11	July 28	Oct. 7	25	6	183.1	102.5	78.0	44.1	13.3	42.1	Oct. 16	25	7	297.1	197.1	75.7	47.0	36.8	16.2
		Oct. 9	30	8	200.0	137.5	87.5	31.3	25.0	38.7	Oct. 23	30	5	330.0	202.0	95.0	38.9	32.4	23.1
		Oct. 14	35	8	145.0	90.0	56.2	38.1	23.2	38.7	Oct. 23	35	5	330.0	202.0	95.0	38.9	32.4	23.1
		Oct. 23	35	8	145.0	90.0	56.2	38.1	23.2	38.7	Oct. 23	35	5	330.0	202.0	95.0	38.9	32.4	23.1
		Oct. 30	40	6	215.0	121.6	73.9	43.5	21.3	35.2	Nov. 8	40	5	314.0	248.0	155.0	21.1	29.6	49.3
		Sept. 20	5	11	80.0	13.6	6.8	83.0	8.5	8.5	Nov. 8	5	10	241.0	112.0	26.0	53.7	35.6	10.7
		Sept. 27	10	11	94.2	27.1	12.5	71.3	15.4	13.3	Oct. 25	10	6	391.0	280.0	141.6	28.6	35.3	36.1
		Oct. 4	15	14	85.3	25.0	12.5	50.0	26.8	14.2									
		Oct. 11	20	14	135.0	54.6	19.2	50.5	35.3	14.2									
		Oct. 17	25	8	160.0	80.7	20.0	53.2	34.3	12.5									

Records lost.

Small.

The results obtained in the first seven plantings show fairly close agreement, no outstanding variations appearing in the corn from these plots. The first significant differences are observed in the corn of the eighth planting of Stowell's Evergreen and the ninth planting of Golden Bantam. Here there is noted a distinct falling off in the yield in the corn 20 days and over in age, caused by the slower rate at which maturity progressed, and the differences are even more marked in the corn of the later plantings. For instance, in the tenth planting of Stowell's Evergreen the yield of cut corn from ears 50 days old was scarcely equal to that of the earlier planting at 30 days

of age. Comparable differences are noted in the late plantings of Golden Bantam.

Reference to the figure giving the meteorological data will show that this falling off in yield is closely correlated with the temperature conditions prevailing during the growing season of these late plantings.

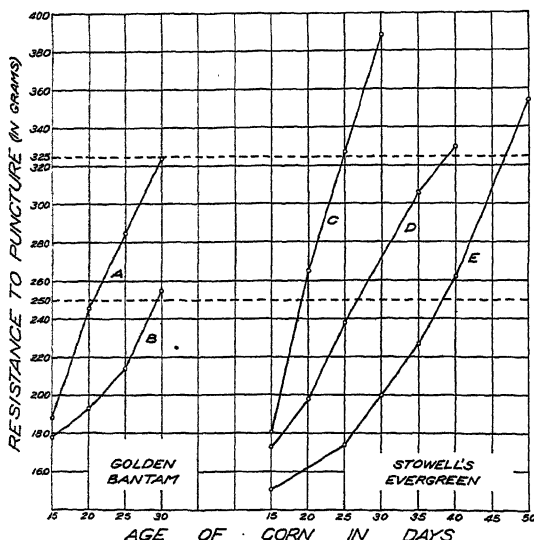


FIG. 5.—Degree of toughness of Golden Bantam and Stowell's Evergreen sweet corn at different stages of maturity, as shown by puncture tests made on samples from the different plots. Curve A, average of the first eight plantings; B, average of the ninth, tenth, and eleventh plantings; C, average of the first seven plantings; D, results from corn of the ninth planting; E, results from corn of the tenth planting

TENDERNESS

The quality of canned sweet corn is more closely associated with tenderness of the kernels than with any other property of the raw corn, and it is of interest, therefore, to observe the effect of seasonal

conditions on the degree of toughness which the kernels possess.

By the use of the puncturing instrument described by the writers in an earlier paper (3), toughness tests were made on the corn from all the test plots at different stages of maturity, and the results of these tests are assembled in Figure 5. The ordinates show the amount of pressure, expressed in grams, required to puncture the kernels with a blunt-pointed needle constructed from No. 16 gauge brass wire, and the abscissas show the age of the corn in days when the tests were made. Because of the close agreement of results with the corn of the first eight plantings of Golden Bantam, the data for these are combined in a single curve. The results from the first seven plantings of Stowell's Evergreen, for the same reason, are similarly expressed.

It is noted that those curves showing the average results from the first plantings of both Golden Bantam and Stowell's Evergreen rise very rapidly, closely approximating straight lines, and that the curve

for Stowell's Evergreen is somewhat steeper than that for Golden Bantam. The degree of toughness reached is also considerably higher. Of particular interest, however, are the curves illustrating the findings on the corn of the later plantings. Here it is seen that the toughening process was considerably retarded, and that with succeeding late plantings, as shown in the curves for Stowell's Evergreen, the retardation became greatly marked.

In the earlier studies it was found that, taking into consideration the excessive toughness of over-mature corn, on the one hand, and too thin consistency due to immaturity, on the other, corn grading in resistance to puncture between the limits of 250 and 325 gm., as measured by this method, yielded a product of the highest quality. In these tests Golden Bantam did not exceed the upper limit for toughness in any of the plots under 30 days of age. On the other hand, the first seven plantings of Stowell's Evergreen passed through the prime canning stage, as judged by the toughness factor, at 19 to 25 days, the ninth plot at 27 to 39 days, and the tenth plot at 38 to 47 days of age.

While it is true that different varieties of sweet corn, and even different strains within the same variety, normally show unequal degrees of toughness at comparable stages of maturity, it is seen that seasonal factors also very definitely affect the tenderness of the kernels. In the present case the widest differences are noted between those plots of corn maturing during the hottest part of the season, on the one hand, and those maturing during the cooler weather of the late fall, on the other; and it would appear from this that temperature is the most important factor concerned with this phenomenon.

CHEMICAL STUDIES

The development of the corn kernel has its morphological, physiological, and chemical aspects. These are interrelated and each has an important bearing on the quality of the canned product. The complete chemical story is infinitely complex, owing to the working out of manifold vital processes, and only the gross chemical changes can be followed in the development of the kernel to maturity. However, the accumulation and transformations of the carbohydrates are so great that they are easily followed. Their significance as nutritive materials and the rôle which they play in determining quality in the canned product are of such importance that it seemed wise to undertake a rather thorough study of them in the present work.

As already stated, samples were taken for chemical analysis at 5, 10, 15, 20, 25, and 30 days from the date of silking from each of the plantings. The methods of analysis were practically the same as those used by the writers in their previous study (3).

MOISTURE

In the above-mentioned publication it was shown that for any variety the degree of maturity was rather closely correlated with the moisture content, the lower the moisture content the more mature the corn. From the present work it is apparent that this also holds true, regardless of the time when the plantings are made. In Table 2 are given the figures for total solids found in Golden Bantam and Stowell's Evergreen corns from each of the experimental plots, sampled at different stages of maturity.

TABLE 2.—Percentage of total solids in Golden Bantam and Stowell's Evergreen sweet corn at different stages of maturity, from plantings made at intervals throughout the season

Age of ears in days	GOLDEN BANTAM										
	Planting										
	1 Apr. 28	2 May 5	3 May 10	4 May 23	5 May 31	6 June 7	7 June 17	8 June 27	9 July 9	10 July 18	11 July 28
5.....	10.62	13.11	12.02	11.83	12.56	13.41	9.93	10.51	10.36	10.54	9.66
10.....	12.56	13.18	13.96	13.76	11.67	13.56	11.21	10.21	10.01	9.99	10.01
15.....	20.67	19.99	19.78	22.28	22.51	19.81	18.64	18.59	12.59	10.68	10.77
20.....	30.18	33.87	31.95	32.19	29.60	28.12	29.09	26.50	15.05	13.21	12.60
25.....	37.31		36.70	35.53	35.02	33.35	31.50	28.10	18.58	16.61	15.98
30.....	41.53	41.97	42.83	40.21	39.21	41.22	33.63	30.92	27.38	19.79	18.97
35.....									31.09	21.43	23.21
40.....										27.56	26.46
50.....											32.06
60.....											34.95

STOWELL'S EVERGREEN										
5.....	11.60	10.64	11.72	13.74	12.58	11.40	10.69	9.45	10.86	8.75
10.....	11.28	11.72	11.43	12.78	12.54	12.60	11.56	11.04	10.23	9.12
15.....	16.02	17.97	18.30	17.87	16.47	14.02	14.27	13.55	10.11	9.38
20.....	21.43	20.76	21.03	24.39	20.62	22.01	20.35	17.36	11.70	10.89
25.....	26.04	27.23	26.88	27.37	27.24	25.68	25.08	19.82	15.41	12.00
30.....	31.36	33.95	35.22	35.32	32.33	27.35	28.39	24.33	20.50	15.26
35.....								29.16	23.99	18.69
40.....									26.27	21.87
50.....										25.92
60.....										28.62

It is seen that the moisture content steadily decreased during the development and maturing of the kernel. In the 5 and 10 day samples it did not vary greatly in any of the plantings, the total moisture amounting to 86 to 90 per cent. The moisture in the corn at the 15-day stage did not vary much for the first eight plantings, but the ninth, tenth, and eleventh plantings showed a marked increase resulting from the decreased rate of maturing. At the 20-day stage the first seven plantings showed only small variations, but the eighth increased considerably, and the ninth, tenth, and eleventh showed a progressive increase. At the 25 and 30 day stages the variation was not great in the first six plantings, but a progressive increase was shown in the corn of all the later plantings. These changes are best shown in Figure 6, which illustrates by curves the differences in the total solids of the kernels of the different plantings at various stages in their development.

By correlating the data on moisture in the corn with the weather records shown in Figure 1, it becomes apparent that the primary factor affecting the moisture changes in the developing ears was temperature, for all samples taken on or after September 10 showed an increase in the moisture content. The sudden change early in September from summer to late autumn temperatures, which has already been mentioned, caused a rather abrupt slowing down in the rate at which the corn matured, and the moisture content of the kernels at different stages of development was higher than that of corn of like age sampled previously. A sudden change in temperature produces a very noticeable effect in a few days.

It is noted that throughout the entire series of tests Stowell's Evergreen had a higher moisture content than Golden Bantam, being especially marked after the 15-day stage. A high moisture content seems to be a specific characteristic of Stowell's Evergreen,

and appears to be correlated with its somewhat slower rate of maturing as observed in the field. However, the writers have been led to doubt whether the difference in the moisture content of different varieties is a true measure of the differences in the rate of maturity.

The moisture content of sweet corn very greatly affects the consistency of the canned product. At the 15-day stage the corn is entirely too watery to make a satisfactory product, while at the 30-day stage it is entirely too dry. The difference in the moisture content of Stowell's Evergreen and Golden Bantam results in a difference in the consistency of the canned material from the two varieties. The 20-day Golden Bantam is of very nearly the same consistency as the 25-day Stowell's Evergreen, and from the table

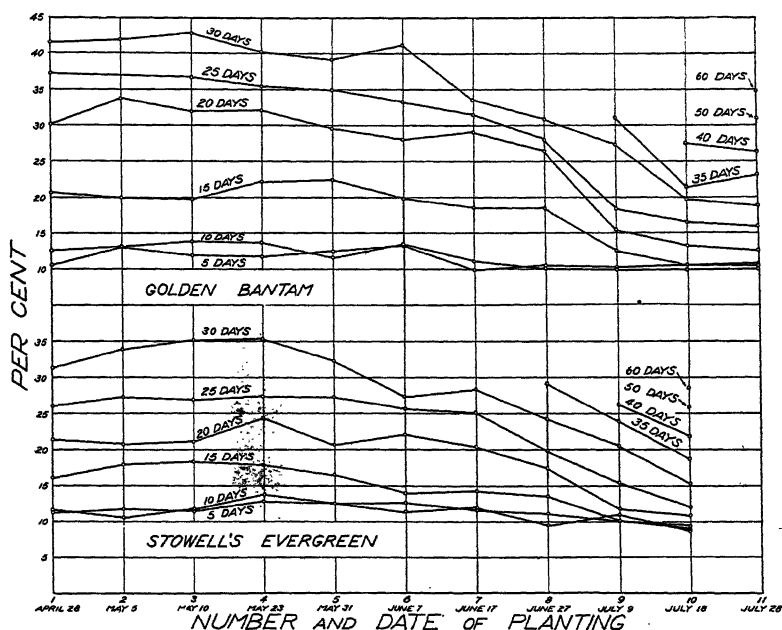


FIG. 6.—Total solids in the cut corn from ears of Golden Bantam and Stowell's Evergreen sweet corn harvested from the different plots at different stages of maturity

given above it is observed that the moisture content is very nearly the same in the two cases.

SUGARS

During the development and maturing of the sweet-corn kernel the sugar content is constantly changing. The percentage present is determined by its concentration in the cell sap and the relative proportion of cell sap to accompanying insoluble constituents. Some of the young samples have a rather high sugar content caused in part by the absence of other substances, such as starch and dextrin. The ratio of the solids to sugar is therefore important, and since the polysaccharides increase tremendously in the older corn the lower percentage of sugar in these is due in part to an increase in polysaccharides rather than to a change in concentration. The percentage of sugars found in the corn at different stages of growth in the various plantings is shown in Table 3.

The changes that took place during the development and maturing of the kernels were nearly the same for all plantings in spite of rather wide variations in climatic conditions. However, the rate at which the changes occurred was tremendously affected by the prevailing temperature. The history of the total sugars began with a medium percentage of sugar, which increased for a time and then decreased as complete maturity approached. The reducing sugars were high in the early stages of development and steadily decreased as maturity advanced. The sucrose was low at first, increased rapidly for a time, and then decreased steadily as the corn matured. These changes are shown in detail in Figures 7 to 10.

The quantity of sugars present was rather uniform for the first seven plantings, always being highest in the corn at the 15-day stage.

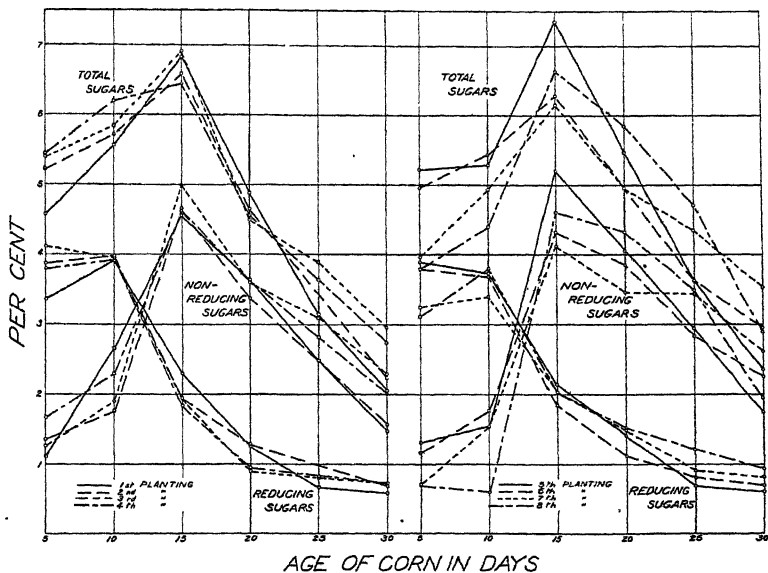


FIG. 7.—Sugar content of the cut corn from ears of the first eight plantings of Golden Bantam sweet corn sampled at different stages of maturity

In the later plantings the highest point was reached at an age greater than in the first plantings, with the temperature as the principal factor affecting the variation. There was no very great change in the ratio of the sugars to each other.

A small but significant difference was apparent in the ratio of total sugars to the total polysaccharides between the early plantings and those made very late, there being a greater proportion of sugar as compared with polysaccharides in the late plantings that matured in the very cool part of the season. Where methods of handling are the same, this greater proportion of sugar in the corn maturing in the cool season should result in a superior canned product. Such an effect was observed in the corn canned from the late plantings in the present experiments, though the improvement in quality was not great. It should be remembered that the greatest effect of cool weather on corn is to slow down the rate at which maturity proceeds.

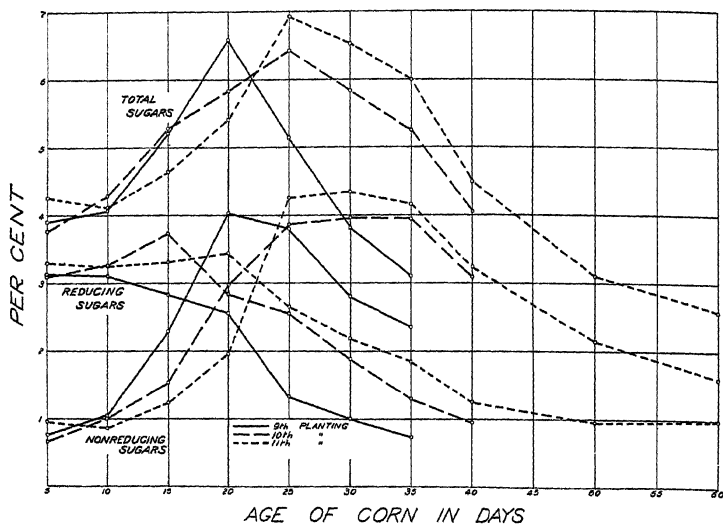


FIG. 8.—Sugar content of the cut corn from ears of the ninth, tenth, and eleventh plantings of Golden Bantam sweet corn sampled at different stages of maturity

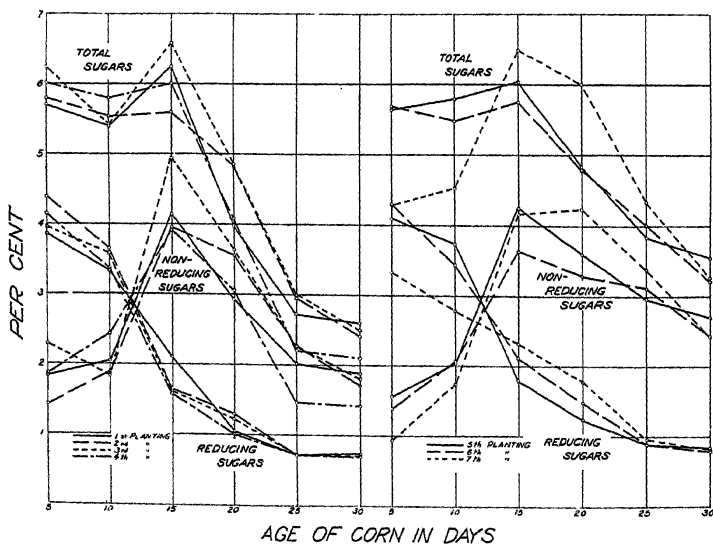


FIG. 9.—Sugar content of the cut corn from ears of the first seven plantings of Stowell's Evergreen sweet corn sampled at different stages of maturity

The rate of change in sugar content is very different from the rate of change in the tenderness of the corn, the two processes being, apparently, very largely independent of each other. The toughness increases very rapidly as the corn gets older, but the sugar content changes less rapidly.

The figures show that Golden Bantam has a slightly higher sugar content at comparable stages of maturity than Stowell's Evergreen.

POLYSACCHARIDES

The polysaccharides in sweet corn seem to be a complex mixture of substances which are chemically or physically different. Approximately one-half of the total amount present in the corn at canning maturity is made up of a water-insoluble portion, which qualitative

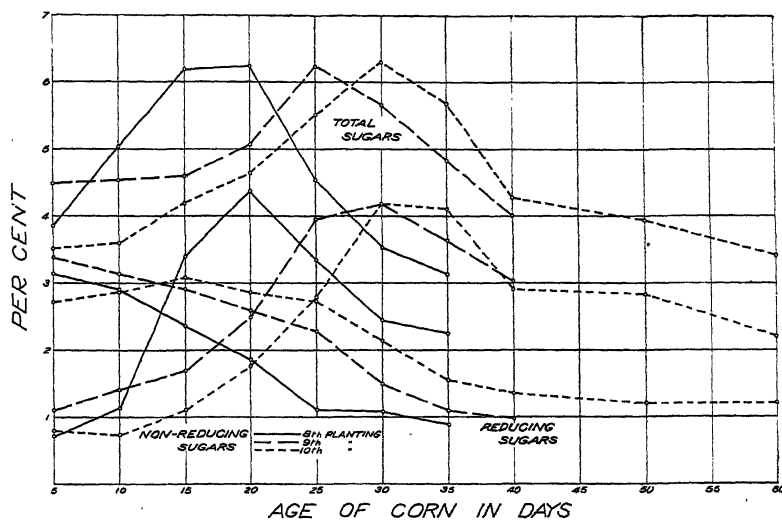


FIG. 10.—Sugar content of the cut corn from ears of the eighth, ninth, and tenth plantings of Stowell's Evergreen sweet corn sampled at different stages of maturity

chemical tests and microscopic examination indicate is composed principally of starch. The other, or water-soluble fraction, usually shows the presence of two substances, one giving a red color reaction with iodine solution, and the other a blue. The nature and proportion of these various substances making up the total polysaccharides have been given particular attention by Lampe and Meyers (7).

From the standpoint of both the canner of sweet corn and the consumer of the canned product the polysaccharides are of great importance, for they are the primary constituents of the corn which affect the consistency of the canned material and comprise the major portion of the nutritive substances. Starch gives to corn a viscous, heavy consistency, while the water-soluble polysaccharides make the canned product more creamy and palatable.

In Table 4 are given the figures for total polysaccharides, calculated as starch, obtained by analysis of the corn from the different plantings harvested at different stages of maturity.

TABLE 4.—Percentage of total polysaccharides, calculated as starch, in Golden Bantam and Stowell's Evergreen sweet corn at different stages of maturity, from plantings made at intervals throughout the season

GOLDEN BANTAM											
Age of ears in days	Planting										
	1	2	3	4	5	6	7	8	9	10	11
	Apr. 28	May 5	May 10	May 23	May 31	June 7	June 17	June 27	July 9	July 18	July 28
5.....	1.68	1.84	2.02	2.12	2.22	2.42	1.70	1.90	1.87	1.68	1.65
10.....	1.88	1.97	2.11	2.13	1.86	2.40	1.82	1.68	1.66	1.62	1.76
15.....	7.97	8.41	8.86	9.73	8.12	7.98	7.37	7.30	2.10	1.26	1.79
20.....	17.79	19.80	19.96	19.59	16.98	16.61	17.16	14.54	4.32	2.48	2.26
25.....	26.19		24.81	24.62	23.80	22.17	20.51	17.59	8.07	5.33	4.59
30.....	30.58	29.31	30.48	28.33	27.47	29.60	22.26	20.36	16.74	8.48	8.14
35.....									19.86	11.76	11.42
40.....										17.42	15.92
50.....											20.37
60.....											24.77

STOWELL'S EVERGREEN											
5.....	1.68	2.08	1.60	2.32	2.02	1.78	1.79	1.50	1.43	1.45	-----
10.....	1.60	1.68	1.75	1.96	1.97	2.30	1.71	1.65	1.53	1.49	-----
15.....	5.54	6.77	6.86	6.93	6.31	4.16	4.22	3.98	1.42	1.43	-----
20.....	12.26	10.98	10.94	11.86	11.07	11.94	10.11	7.24	3.12	1.61	-----
25.....	16.96	17.43	17.25	17.73	16.63	15.48	15.40	10.35	4.83	2.25	-----
30.....	21.97	23.70	24.90	24.40	20.40	18.14	18.72	14.90	9.73	4.93	-----
35.....								19.32	13.72	8.69	-----
40.....									16.20	13.24	-----
50.....										16.52	-----
60.....										19.94	-----

It is seen that during the development and maturing of the sweet corn kernel the polysaccharide content increased steadily. The quantity present in corn of the same age in the first seven plantings did not vary greatly, but the rate at which it accumulated in the corn of later plantings fell off rapidly as the season advanced. A change in the moisture-polysaccharide ratio was therefore observed.

As noted in the case of the total solids, there was found a somewhat greater proportion of sugar to total polysaccharides in the corn of the late plantings than in that of the earlier plantings.

The story of the polysaccharide changes as observed in the present experiments is illustrated in the curves of Figure 11.

DEXTRIN

The water-soluble polysaccharide giving a red color reaction with iodine solution appears to be a dextrin, or a closely related substance, and since it comprises the major portion of the water-soluble fraction, the figures for total water-soluble polysaccharides given in Table 5 are calculated as dextrin, although there was more or less material present giving a blue coloration with iodine.

By comparing the figures of this table with those for total polysaccharides in Table 4 it is seen that in the early stages of development the proportion of the water-soluble fraction was much less than in the older corn. No significant effect of seasonal factors on the ratio of water-soluble to total polysaccharides, however, was found.

Curves showing the history of the water-soluble polysaccharides in these experiments are given in Figure 12.

CANNING TESTS

From the practical standpoint, the quality of the corn in the can is the most important consideration in studies of this sort. As before stated, canning tests were made upon the corn of each plot at the ages of 15, 20, 25, and 30 days. After husking, trimming, and washing, the corn was cut from the ears by hand machine, the knives

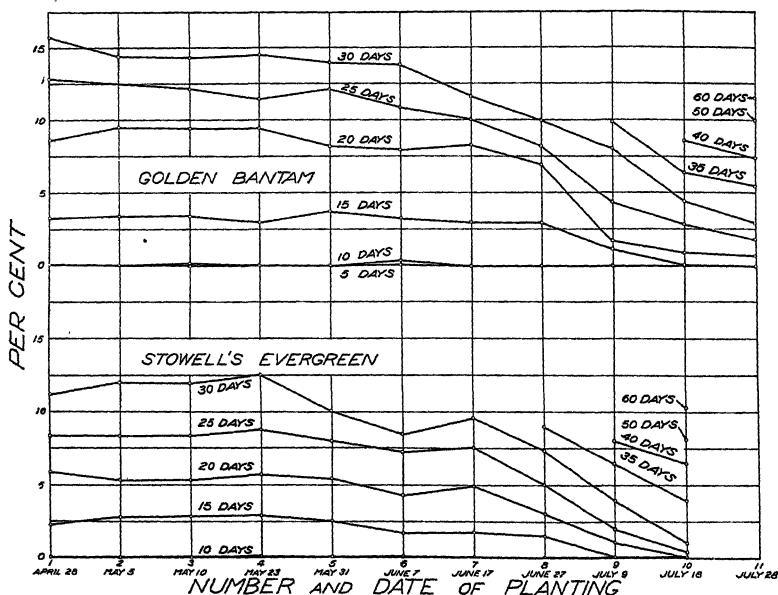


FIG. 12.—Water-soluble polysaccharide content of the cut corn from ears harvested from the different plots of Golden Bantam and Stowell's Evergreen sweet corn at different stages of maturity

being arranged so as to remove the outer one-half to two-thirds of the kernel, and the remainder of the kernel contents was scraped from the cob by the mechanical scrapers of the machine. The cut corn was then weighed and enough brine (containing 2 per cent salt and $6\frac{1}{4}$ per cent sugar) added to it to give the ratio of cut corn to brine, by weight, of 4 to 1.

The corn was then precooked in a steam-jacked cooker provided with a mechanical stirring device, and the cooking continued, with constant stirring, until the temperature reached 85° to 90° C. It was filled into the cans immediately, the cans were sealed, and the processing performed promptly. The procedure was the same for each lot of corn.

By following a uniform procedure in the canning tests it was possible to observe any variations in quality in the canned product due strictly to seasonal factors.

In judging the quality of the cut-out corn, attention was given particularly to tenderness, consistency, flavor, and sweetness. The results obtained follow.

GOLDEN BANTAM

In the first seven plantings the corn showed no significant differences at the various stages. Prime canning condition was found to center around the 20-day stage, the 15-day corn being too immature and the 25-day corn too far advanced for best quality. The 25-day corn of the seventh planting, however, was not quite so mature as that from those immediately preceding. The corn from the eighth plot was almost prime at the 25-day stage, though a very good product was obtained from the 20-day corn. There was observed here a slightly lengthened period during which first-quality canned corn could be produced. In the product from the ninth planting the corn was found to be too immature at 25 days of age and about prime at the 30-day stage. The corn from the tenth planting was slightly too immature at the 30-day stage, being in about the same condition at this age as the corn of the first plot at the 15-day stage of maturity.

STOWELL'S EVERGREEN

In the case of this variety the corn of the first four plantings showed no significant differences in the quality of the products from corn of like age. The 15-day corn was tender and sweet, but the product was watery and the corn too immature to make possible the packing of a product of first quality. At the 20-day stage the corn was slightly under prime, from the standpoint of consistency, but was sweet and tender. At 25 days the kernels had toughened perceptibly and the natural sweetness had largely disappeared, but the consistency was good. The corn 30 days old was tough and starchy, being well past the canning stage.

Prime canning maturity in the corn from the first four plantings, therefore, was found to be close to but slightly beyond the 20-day stage.

In the corn from the fifth planting a slight slowing down in the rate of development was noted. At the 20-day stage the product was tender and sweet but somewhat lacking in body, while the 25-day corn was nearly prime. At the 30-day stage the corn yielded a product of fair consistency, but the kernels were tough. In the case of the sixth planting the findings were similar to those just described for the fifth, the corn being in prime canning condition at about the 25-day stage.

The cut-out material from the seventh planting showed a slight further slowing down in the rate of maturing, in this case the 25-day corn being at the lower limit of maturity for prime consistency. At 30 days of age the corn was sweeter than that at the 25-day stage of the first four plantings, and though a little tough, was satisfactory from the standpoint of consistency. In the material from the eighth plot the corn at the 30-day stage was sweet and of about the same consistency as that of the first four plantings at 20 days. The corn of the ninth planting was even less mature at comparable stages, that 30 days old yielding a product that was too watery and immature for first quality.

The corn of the tenth planting was utilized in the study of frost injury, the findings of which will be discussed presently.

It will be seen from these observations, therefore, that the period during which the ear of corn develops has a very great deal to do with the rate at which maturity progresses and, accordingly, with the quality of the canned product. The correlation of these findings with the data of related studies and with the seasonal factors themselves will be undertaken in the general discussion which follows the presentation of experimental results.

FROST INJURY

It is commonly considered by sweet-corn canners that if corn becomes "frost bitten" it is seriously injured for canning purposes, and a light frost is thought to cause considerable deterioration in the quality of the corn. So far as the writers have been able to learn, almost no work has been published on the effect of frost injury upon the quality of canned corn. Bushey (2) and Spitzer and his associates (10) have made studies upon the composition of soft corn (field corn), but their results are hardly comparable with those given here, as most of their analyses compare thoroughly ripened corn with corn more or less immature. A higher moisture content and larger quantities of amide nitrogen were generally found. Kiesselbach and Ratcliff (6) have shown that freezing the immature corn injures it for seed purposes, and it is also injured if there is a high moisture content.

The plantings in the present experiments furnished material admirably suited to a study of the effect of frost. By going through the last plantings of each variety one could obtain samples at almost any stage of maturity.

On the night of October 21 a light frost occurred which resulted in the killing of approximately two-thirds of the leaf area of most of the leaves. The bases of the blades and the stalks and ears were not injured materially. Canning tests were made on the afternoon of the twenty-first on corn from ears of Stowell's Evergreen 30 days and also 35 days old, and likewise from ears of Golden Bantam 30 days old. The tests were repeated two days later. Chemical samples were also taken. In an examination of the cut-out material no difference whatever could be found in the corn canned before as compared with that canned after the frost, unless perhaps a slight difference in consistency, which was to be expected of corn two days older. The results of the chemical analyses shown in Table 6 indicate very little alteration in the chemical composition, and nothing that could be attributed to frost injury. The normal ripening processes continued, but at a much slower rate because of the lower prevailing temperature.

TABLE 6.—Percentage composition of Stowell's Evergreen and Golden Bantam sweet corn before and after frost

Variety	Date of sampling	Total solids	Total polysaccharides as starch	Water soluble polysaccharides as dextrin	Sugars, as invert		
					Total	Reducing	Non-reducing
Stowell's Evergreen, 30 days old.	Oct. 21	18.84	6.66	2.94	6.11	1.75	4.36
	Oct. 23	17.03	7.29	3.11	5.98	1.57	4.41
	Oct. 21	22.14	12.07	5.68	5.02	1.57	3.45
Stowell's Evergreen, 35 days old.	Oct. 23	23.27	13.29	6.25	4.92	1.29	3.63
	Oct. 21	16.16	5.83	2.10	6.98	2.30	4.68
Golden Bantam, 30 days old....	Oct. 23	16.42	5.88	2.15	6.82	2.43	4.39

During the period following the frost of October 21 up to the 16th of November the corn continued to mature, though at a very slow rate, which affected the canned samples correspondingly.

On the night of November 16 a heavy freeze occurred in which the stalks and ears were completely frozen. Canning samples were taken early in the morning after the freeze and again five days later. Corn of two stages of maturity was used, one lot from ears 50 days old and the other from ears 60 days old, these being not far removed either way from prime canning maturity.

The quality of the corn in the cut-out samples showed much less difference in the quality of the product than was expected. The corn canned on the morning after the freeze was normal in appearance and flavor. That canned five days later showed a little browning, which was apparently caused by the oxidation of tannins in the cob particles removed with the corn in cutting, and the appearance of the product was, therefore, not quite so attractive. There was a slight "cobby" flavor to the corn, but this was not very marked, and had one not known the history of the corn it probably would have been consumed without much hesitation.

Chemical samples were taken on the morning after the freeze and at intervals thereafter. Table 7 shows the results of the analyses.

TABLE 7.—Percentage composition of Stowell's Evergreen and Golden Bantam sweet corn sampled immediately and at intervals after complete freezing

Variety	Date of sampling	Total solids	Total polysaccharides, as starch	Water soluble polysaccharides, as dextrin	Sugars, as invert		
					Total	Reducing	Nonreducing
Stowell's Evergreen, 50 days old.	Nov. 17	22.62	12.68	5.98	4.90	1.26	3.64
	Nov. 22	23.53	13.67	7.07	4.56	1.51	3.05
	Nov. 27	24.35	13.69	7.08	5.12	2.26	2.86
	Dec. 2	22.32	12.81	6.68	4.97	2.54	2.43
	Dec. 7	22.96	12.52	6.51	4.43	2.90	1.53
Stowell's Evergreen, 60 days old.	Nov. 17	30.03	19.94	10.32	3.42	1.22	2.20
	Nov. 22	29.06	18.29	9.24	3.33	1.58	1.75
	Dec. 2	28.62	17.76	9.19	3.64	2.01	1.63
	Dec. 7	29.55	19.58	9.62	3.98	2.32	1.66
	Nov. 17	30.33	18.64	9.21	3.90	2.27	1.63
Golden Bantam, 50 days old....	Nov. 27	31.54	19.60	9.66	3.69	3.09	.60
	Dec. 2	31.28	19.08	9.02	3.04	2.50	.54
	Nov. 17	34.95	23.92	11.26	3.58	.98	2.60
Golden Bantam, 60 days old....	Nov. 27	35.29	24.77	11.60	2.88	1.64	1.24
	Dec. 2	35.75	23.52	11.16	3.73	2.28	1.45

The results were somewhat irregular because of errors in sampling. The moisture content did not change very materially during the entire time, though there were indications of a slight decrease. The total polysaccharides behaved in about the same way, increasing when the moisture decreased, and vice versa. There appeared to be a very marked tendency for the reducing sugars to increase and the sucrose to decrease. It is not certain, however, that all the increase in reducing sugars arose from the inversion of sucrose.

The climatic conditions subsequent to the freeze were not such as to cause much drying or rapid fermentation of the corn. After five days the cob and chaffy material at the base of the grains began to turn brown, and soon afterwards the grain itself became somewhat discolored. This rendered it unfit for canning.

The rate at which deterioration will occur after the corn is frozen depends upon climatic conditions. If it is very warm, completely frozen material will discolor more rapidly and fermentation may take place. Practically no fermentation occurred in the corn in these tests. Between 10 and 15 days after the freeze an occasional ear was found which was invaded by molds. After 15 days the number increased rapidly, and in time practically all the remaining ears were spoiled in this way.

GENERAL DISCUSSION

To assist in the correlation of seasonal conditions with the variations observed in the field plots and in the corn derived therefrom Table 8 has been prepared. This table indicates in dates and days the progress of growth and maturity of the corn from the different plots. The times specified under "Date of silking" were the mid-silking dates, or those days on which the peak of silking occurred, as determined by the daily tagging records, and in the column showing the time at which the corn came to canning maturity corresponding dates are given. The exact dates, in this latter case, were derived by adding to the date of silking the number of days required thereafter for the corn to arrive at canning maturity, as shown in the last column of the table. The data of this last column were secured by a study of two factors, namely, the quality of the cut-out corn from the practical canning tests and the moisture content of the raw corn as determined by chemical analysis, which were in close agreement.

TABLE 8.—*Effect of seasonal conditions on the development and maturing of Golden Bantam and Stowell's Evergreen sweet corn at Arlington Experiment Farm during the season of 1924*

GOLDEN BANTAM						
Number of planting	Date of planting	Date of silking	Days from planting to silking	Date of canning maturity	Days from planting to canning maturity	Days from silking to canning maturity
1.....	Apr. 28	July 16	79	Aug. 5	99	20
2.....	May 5	July 21	77	Aug. 10	97	20
3.....	May 10	do.....	72	do.....	92	20
4.....	May 23	July 23	61	Aug. 12	81	20
5.....	May 31	July 30	60	Aug. 19	80	20
6.....	June 7	Aug. 3	57	Aug. 23	77	20
7.....	June 17	Aug. 11	55	Aug. 31	75	20
8.....	June 27	Aug. 21	55	Sept. 13	78	23
9.....	July 9	Sept. 2	55	Oct. 2	85	30
10.....	July 18	Sept. 14	58	Oct. 24*	98	40
11.....	July 28	Sept. 24	58	-----	-----	-----
12.....	Aug. 9	Oct. 13	65	-----	-----	-----
STOWELL'S EVERGREEN						
Number of planting	Date of planting	Date of silking	Days from planting to silking	Date of canning maturity	Days from planting to canning maturity	Days from silking to canning maturity
1.....	Apr. 28	July 29	92	Aug. 21	115	23
2.....	May 5	July 31	87	Aug. 23	110	23
3.....	May 10	Aug. 3	85	Aug. 26	108	23
4.....	May 23	Aug. 4	73	Aug. 27	96	23
5.....	May 31	Aug. 11	72	Sept. 3	95	23
6.....	June 7	Aug. 14	68	Sept. 8	93	25
7.....	June 17	Aug. 19	63	Sept. 13	88	25
8.....	June 27	Aug. 29	63	Sept. 29	94	31
9.....	July 9	Sept. 15	68	Oct. 24*	107	39
10.....	July 18	Sept. 25	69	-----	-----	-----
11.....	July 28	Oct. 16	80	-----	-----	-----

* This was the date on which the peak in canning maturity would have been reached had not the frost on the night of Oct. 21 occurred. The data on which this statement is based were obtained from ears tagged before the peak of silking was reached, which were sampled before Oct. 21.

Considering first the data concerned with Golden Bantam, it will be noted that the length of time required for the plantings to come to the peak of silking ranged from 79 to 55 days, an extreme variation of 24 days. The most rapid development was recorded in plots 7, 8, and 9, which were planted on June 17 and 27 and July 9, respectively. In the tenth planting there was a distinct lengthening of the growing period.

In the figures for the days required for the corn to reach canning maturity it is seen that the time ranged from 99 days in the first planting to 75 days in the seventh, beyond which the time increased rapidly to 98 days in the tenth.

With respect to the length of the period between silking and canning maturity no difference was found in any of the plots planted up to June 17, the period in all being 20 days. In the eighth planting a little lengthening of the period was noted, and in the ninth the time was increased to 30 days. Forty days were required for the corn of the tenth plot to arrive at canning maturity after silking.

In the data for Stowell's Evergreen similar conditions are observed. Here the length of the period from planting to the peak of silking ranged from 92 days in the first plot to 63 days in the seventh, an extreme variation of 29 days. The length of this period for the seventh and eighth plots remained stationary at 63 days, but beginning with the ninth a distinct lengthening was observed, which increased more and more up to the eleventh planting, when the length of the period was 80 days.

Similar results were obtained with respect to the length of time from planting to midcanning maturity in the different plots. In the first planting this period amounted to 115 days, decreasing in succeeding plantings to 88 days in the seventh and lengthening again in the eighth and ninth.

The figures giving the length of the period from silking to canning maturity in Stowell's Evergreen show results similar to those observed in Golden Bantam. Here, however, the period was 23 days for the first five plantings, the succeeding planting showing a progressive lengthening up to 39 days in the ninth.

It is seen, therefore, that both varieties, though very different in most of their characteristics, responded in an identical manner to certain external influences. A careful analysis of the data presented here shows that in all those variations where there was a distinct change toward a longer growth and maturing period the time at which the change occurred in both varieties was in the early days of September. Thus in the case of Golden Bantam the period of time between planting and silking began to increase at between September 2 and September 14, the length of the period from planting to canning maturity began to increase between August 31 and September 13, and the period from silking to canning maturity showed an increase at the same time.

In the Stowell's Evergreen the period from planting to silking began to lengthen at between August 29 and September 15 and the period from silking to canning maturity at between September 3 and September 8. The period from planting to canning maturity began to lengthen at between September 13 and 29, later than at first would appear consistent with the foregoing figures, but not

inconsistent when it is realized that the growing period prior to silking in this case occurred during the most favorable growing season, thus offsetting in the total period the lengthening of the time between silking and canning maturity, as indicated in the figures of the final column.

It would appear that the prime factor affecting the rate of maturing in these plots of corn was that of temperature, for with the gradually rising seasonal temperature there was a progressive shortening of the growing and maturing periods and a distinct lengthening of these periods at the time of abrupt change from summer to late autumn temperatures taking place early in September. The constant length of the period from silking to canning maturity in the first seven plantings of Golden Bantam and the first five plantings of Stowell's Evergreen corresponds to the interval of time from July 16 to September 3, which, as reference to the curves of Figure 1 will show, had an unusually uniform summer temperature.

In the previous paper, to which reference has already been made, attention was called to the fact that variations in the time of maturing of different varieties was due to the length of the growing period of the plant—that is, from the time of planting to the time of silking rather than to the length of the period from silking to canning maturity, which was fairly uniform for all varieties. Hopper (5) has made the same observation. While in the present study this held true for the plantings made at the usual season, it did not hold for the later plantings, and future interpretation of experimental results along this line will need to take seasonal factors into consideration.

Change in temperature is quickly reflected both in the development of the plant and in the rate at which the sugar, starch, and other constituents are stored in the kernels. Sudden lowering of the temperature while the plant is still small results in an immediate slowing down in the rate of growth; coming during the flowering season, the tasseling and silking are promptly delayed, the normal processes being resumed upon the return of favorable temperature; coming during the maturing period of the ear, the enzymatic activities are retarded and the development of the kernels is checked; all of which go to show that temperature conditions are intimately related to the vital functions of the sweet-corn plant.

Referring again to the results of the chemical analyses, we find further evidence in support of this view. The relation of temperature to the moisture content of the corn has already been emphasized. In the data showing the transformations in the amount and proportions of the different sugars in the corn of the different plots it is found that the first significant variations from the figures for the corn of the early plantings occur in those samples taken after the pronounced temperature change beginning in early September had affected the rate of maturing of the corn. Like effects are observed in the figures on polysaccharides, and confirmatory evidence is also found in the data on the rate of increase and degree of toughness in the corn of the various plots.

One fact of practical significance which has been brought out in these studies on the relation of temperature to quality is that because of the slowing down in the rate at which maturity in sweet corn proceeds during the cooler season there is a longer period during which a

first-quality product can be canned, the corn remaining in canning condition considerably longer than in the case of that maturing during the hot season. This has been emphasized in the discussion concerning increase in toughness, but the results of chemical analyses point to the same fact.

The rôle played by rainfall in the vital processes of sweet corn has been the subject of particular study in the present investigations, and its very important relation to the vegetative activities of the plant has already been discussed. One might expect to find also in the chemical transformations taking place within the developing corn kernels a very close relation between soil moisture and the chemical composition of the kernel constituents, which would be reflected first of all in the moisture content of the corn kernels, with corresponding variations in the other chemical constituents. Care was taken to check over all analyses to discover any correlation between the moisture content of the corn samples and the drought and rainfall conditions of the season.

The drought conditions that prevailed during the last of July and the first 12 days of August, however, did not affect appreciably the moisture content of the samples taken during this period. The month of October was also practically rainless, but the temperatures were considerably lower than during the drought of July and August, and the evaporation of soil moisture was correspondingly less. There was no decrease in the moisture content of the samples taken during the drought of October; on the contrary, the moisture content of these samples was even higher than in the corn of corresponding age from the earlier plantings, due, evidently, to the decrease in the rate of maturing because of the lower temperatures.

From the experimental evidence presented, it is clear that soil moisture conditions directly affect the vegetative activities of the plant and are reflected particularly in the yield of corn, but while soil moisture does determine to a very large degree the amount of material which the plant is able to manufacture, it does not, apparently, have any appreciable effect on the chemical composition of the kernels up to at least 25 days of age.

Sunshine does not seem to be a limiting factor in the maturing of sweet corn. Its variations are accompanied by other varying factors which complicate the results, so that its effect is uncertain. No general or specific effect has been noted in these studies that could be attributed to it.

Taking into consideration the variations in chemical composition of corn as influenced by age and by environmental conditions, particularly temperature, it is apparent that analytical data on plant products which take no account of these factors are of very limited value.

In the earlier publication, to which reference has already been made, the writers showed that quality in sweet corn is determined primarily by the degree of maturity of the corn when canned. Any influence, therefore, affecting the rate at which maturity proceeds would have a direct relation to the quality of the canned product. It has been shown in the present study that the time required for the corn of the first seven plantings of Golden Bantam to reach canning maturity was the same, and no distinguishable differences in quality

could be detected in the product from corn of the same age. The same was true for the first five plantings of Stowell's Evergreen. In the later plantings where the rate of development was slowed down the corn of the same ages was correspondingly immature, so that to secure the highest quality in the canned product older corn had to be used. In addition to this, however, there was observed in the corn of the late plantings a higher degree of sweetness and tenderness in the corn of prime consistency than was observed in that from the earlier plantings having like consistency, indicating that the corn maturing during the cool autumn temperatures is somewhat superior in quality to that maturing during the warmer season.

The relative merits of the canned product from Golden Bantam and Stowell's Evergreen corn has been the subject of considerable discussion among both canners and users of canned corn. The findings of the present study as bearing on this matter are, therefore, of some interest. It was found that at comparable stages of maturity Golden Bantam was tenderer than Stowell's Evergreen, as determined by kernel puncture tests, and chemical analyses made of the corn at the different stages of maturity showed that the sugar content was, on an average, slightly higher in the Golden Bantam. It is not known that these facts would hold for all strains of these varieties, but they were sufficiently pronounced in the present case to be significant.

Frost as a hazard in sweet-corn canning seems, from the results of the present study, to have been somewhat overestimated. Its seriousness as affecting the quality of the corn in the can is dependent, apparently, on the severity of the frost and the temperature conditions following. In the present case a light frost had no perceptible effect and a heavy freeze made its effect felt only after several days. Mere freezing of the corn does not of itself, so far as could be determined from the samples canned in these experiments, have a deleterious effect on the quality of the cut-out corn.

The sweet-corn grower who desires to use the stover as green feed or for silage is usually anxious that the corn be harvested before it is frosted. There is, of course, a certain amount of loss in succulence of the corn, and it probably is somewhat less palatable to stock, but there is, apparently, no loss in food value as a result of freezing, and except for the loss of some of the material during the harvesting operations caused by its brittle condition after freezing, no particularly harmful effects follow frost injury.

SUMMARY

Seasonal factors, through their influence on the rate of development and maturing of sweet corn, are important in determining the quality of the canned product. In the present experiments the rate of growth, the length of the growing period, the size of the plant and of the ear, the yield of corn, and the rate at which maturity proceeded were modified very materially by variations in the environmental conditions.

The number of days from planting to the time of silking ranged from 55 to 79 for Golden Bantam and from 63 to 92 for Stowell's Evergreen. The principal factor responsible for these variations was temperature. The longest time required to reach the silking stage

was in plantings made early in the spring; the shortest time in plantings made in early summer. As the coolness of autumn approached, the time between planting and silking was correspondingly increased.

The number of days from the time of silking to the time most favorable for canning ranged from 20 to 40. Here also the tests indicated that temperature was the principal factor determining the length of the period. As the maturing period passed into the cool part of the autumn, the most favorable age for canning was correspondingly increased.

The toughness of the corn as measured by its resistance to puncture increased continuously throughout the period of its development. Toughness has a very important influence on the quality of the canned material, for as toughness increases the quality of the corn is progressively lowered until it becomes unsuited for table use. Stowell's Evergreen at corresponding ages was somewhat tougher than Golden Bantam.

The yield of cut corn increased rapidly in the corn maturing in midsummer. In the cool autumn it increased more slowly but for a longer time. The proportion of cut corn to cob and husk continued to increase throughout the entire period of development and maturing of the ears.

The percentage of moisture in the developing grains decreased continuously during the growth and maturing of the ears. It decreased most rapidly during the hot summer days and less rapidly in the late fall. Stowell's Evergreen was found to be characteristically higher in moisture content than Golden Bantam. Corn in prime canning condition had a moisture content ranging from 25 to 30 per cent.

The total sugar content of the very young grains was medium to low, increased for a time as development proceeded, and then decreased as maturity approached. The rate at which the changes occurred depended primarily on the prevailing temperature. Whenever a change in temperature occurred it affected the development of the ear during that period regardless of the stage of maturity.

The sucrose was low in the early stages of development, increased rapidly for a time, and then decreased slowly until maturity was complete. The changes were also affected by the prevailing temperature, so that in the cool autumn the age at which the highest sugar content occurred was greater than in the early plantings.

The reducing sugars were high in the early stages of development of the corn and decreased steadily as maturity advanced, the rate depending on the temperature.

The polysaccharides were found to consist of a portion soluble in cold water, probably chiefly dextrin, and a portion insoluble in cold water, which was principally starch. When the corn was in prime canning condition about one-half of the total polysaccharides were water soluble, and environmental conditions did not seem to alter this ratio to any extent. Owing to the slower rate at which maturity progressed, the quantity of polysaccharides was smaller in the corn developing during the late autumn than in corn of the same age developing during the hot season.

Of the seasonal factors concerned with the growing and canning of sweet corn, temperature was found to be the most important as

affecting the quality of the canned product. The rainfall had a very marked effect upon the vegetative activities of the plant, but produced no significant effect on the chemical composition of the cut corn or the quality of the canned product. No specific effect of sunshine could be detected in the experimental findings of this study.

Light frost had no significant effect on the composition of the corn or the quality of the canned product. For the first few days after a heavy freeze the canning quality was almost equal to that of the unfrozen corn, but the quality deteriorated rapidly thereafter owing to the discoloration and the activities of microorganisms.

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EFFECT OF FEEDING AND MANAGEMENT OF SHEEP ON THE TENSILE STRENGTH AND ELASTICITY OF WOOL¹

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PREVIOUS INVESTIGATIONS

The earlier work on the tensile strength and elasticity of wool at this station has already been reported in a paper in which some of the literature on the subject was reviewed (8).² An improved testing device was described, the observations which should be taken in running tests on wool fibers were demonstrated, and the interpretation of the data from such tests was illustrated. It was shown that breaking stress has a much greater variability than tensile strength, and that the elastic limit and Young's modulus may be more important indications of the value of a set of wool fibers in fabrics than the breaking stress.

Since the appearance of the paper mentioned above, additional information has been published by Hardy on methods of testing wool fibers (4, 5) developing the fact that humidity affects the ultimate strength and the elastic limit of wool fibers.

Types of apparatus for testing wool were described by Henley (6) and Macha (7).

There have been a number of reports showing the effect of feeding and management of sheep on the growth of wool. One of the earlier studies was made by Cooke and Jones (2) in which the feeding to Merino ewes of "carbonaceous" as contrasted with "nitrogenous" rations produced no measurable differences in diameter of fiber as indicated by their results. They point out, however, that the wool fibers of a few of the sheep that "did not do well" were "shrunk in diameter."

Craig (3, p. 18-19) used Shropshire wethers in a study of the relative effects of "carbonaceous" and "nitrogenous" rations. He found that six wethers fed the "nitrogenous" ration clipped 2.4 pounds more unwashed wool and 0.6 pound more washed wool than six wethers fed the "carbonaceous" ration. The additional weight of unwashed wool was credited "in a limited degree, to the amount of yolk in the fleeces of the lots."

Skinner and Smith (10) report a difference of 0.4 pound more wool from ewes fed a dry ration than from those fed a succulent ration (containing corn silage). They do not, however, credit this increase to the difference in the rations.

Feeding either calcium sulphate or a ration high in sulphur contrasted with a ration low in sulphur seemed to have no effect on the gross weight of the fleece, on the percentage of clean wool, on the percentage of sulphur in pure wool fiber, or on the percentage of yolk (9).

¹ Received for publication June 1, 1926; issued December, 1926.

² Reference is made by number (italic) to "Literature cited," p. 1089.

Bray (1) reported some studies of density, breaking stress, and elasticity of fibers of Dorset, Shropshire, Merino, and crossbred sheep.

METHOD OF TESTING THE FIBERS

The method of testing the wool fibers in this study was essentially that described by Miller and Tallman (8). The readings taken were (1) stretch by each increment of weight or stress of 5 dgm., (2) elastic limit point, (3) total stretch at the breaking point, (4) breaking stress, and (5) diameter. The diameters were averages of 10 readings on each fiber. The fibers were mounted in Canada balsam and the readings were taken with a compound microscope.

Small locks of about 25 fibers were separated from the main sample and every fiber in these locks was tested. In this way the longer or more prominent fibers were not tested to the exclusion of the shorter, finer, and less conspicuous fibers. The flesh or inner end of the fiber was placed in one jaw of the testing machine so that the wool grown during the test period was certain to be subjected to the test.

Since facilities were not available for maintaining constant temperature and humidity and especially since the effect of the humidity of the breath and perspiration of the operator could not be eliminated, no attempt was made to control these factors in the testing room. All samples were kept in a desiccator containing calcium chloride for 24 hours before the beginning of the test. Only the small locks were exposed to humid air during the test. With the exception of the earlier work all testing was completed within three months after shearing.

In order to equalize the effect of any tendency to change the routine of the test by the operator, alternate samples from the two lots were tested. The samples were not scoured before testing. The effect, if any, of hardening of the "grease" or "yolk" would also be equally distributed between the lots.

Naturally some phases of the method are more or less arbitrary. The value of the results for comparative purposes depends on the ability of the operator to maintain a constant speed for the various operations. A definite order of procedure was worked out which was designed to occupy his time at every stage of the operation. The measurements of length of staple were taken after shearing. Each value is the average of 6 to 9 measurements of as many locks of wool.

EXPERIMENTAL MATERIAL AND RESULTS

In 1916 a small group of registered Rambouillet ewes from the flock owned by the station was put into a range band and kept under average range conditions for a year. Samples of wool from these ewes were taken in 1917. In 1916 these ewes had raised a few lambs, and samples of their fleeces were also taken in 1917. These samples were compared with those taken from mature ewes and from yearlings ewes and wethers, respectively, that were kept under very good farm conditions during the year.

In 1918 samples were obtained from mature ewes kept under semirange conditions and from ewes kept under very good farm conditions, both lots coming originally from the flock owned by the station.

TABLE 1.—*Tensile strength and elasticity of wool from aged ewes, 1917*

LOT 1

(AGED EWES, IRRIGATED FENCED PASTURE, GOOD FEED IN WINTER, SHELTER)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>Mm.</i>	<i>Dgm.</i>	μ				
392-696...	7.85±0.248	59.60±0.937	15.40±0.115	0.762±0.0081	141,600±1,895	660,000±10,640	0.5589±0.0086
394-859...	3.96±.169	36.50±.809	13.08±.135 ^a	.882±.0135	124,800±3,361	^a 786,600±14,760	.6228±.0158
406-980...	11.88±.297	50.61±.958	12.55±.162 ^b	.801±.0128	186,400±3,194	^b 845,800±18,440	.7067±.0146
476-477...	6.27±.236	59.70±1.148	14.82±.196	.696±.0074	157,900±2,459	789,000±15,400	.6736±.0121
Averages	7.49±0.121	51.60±0.485	13.96±0.077	0.785±0.0054	152,700±1,395	770,300± 7,530	.6405±0.0065

LOT 2

(AGED EWES; RANGE CONDITIONS)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
80-403...	7.00±0.202	48.00±0.910	12.70±0.122	0.890±0.0132	170,000±2,248	839,000±14,000	0.7235±0.0106
221.....	7.04±.310	51.90±1.226	13.52±.155 ^c	.782±.0092	159,300±1,979	^c 831,800±14,020	.6675±.0107
393-961....	7.68±.263	47.50±.674	12.76±.082	.856±.0114	165,100±2,904	834,600±10,930	.7104±.0082
401-847....	8.80±.229	47.85±.843	12.40±.110	.882±.0145	176,600±2,425	914,600±12,510	.7229±.0102
405.....	5.40±.209	55.55±.819	13.56±.138	.787±.0088	175,600±2,740	838,800±15,480	.7552±.0137
404-979....	6.72±.229	52.15±1.012	13.39±.115	.818±.0094	167,800±2,236	828,400±12,150	.7190±.0097
Averages	7.11±0.099	50.49±0.380	13.05±0.050	0.836±0.0046	169,100± 927	847,900± 5,410	.7164±0.0043

^a 88 fibers-12 fibers failed to withstand more than 10 dgm.^b 80 fibers-20 fibers failed to withstand more than 10 dgm.^c 94 fibers-6 fibers failed to withstand more than 10 dgm.TABLE 2.—*Tensile strength and elasticity of wool from yearling wethers and ewes, 1917*

YEARLING WETHERS

(IRRIGATED FENCED PASTURES; GOOD FEED IN WINTER; SHELTER)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>Mm.</i>	<i>Dgm.</i>	μ				
519.....	9.18±0.254	53.30±1.066	14.05±0.118	0.888±0.0133	150,600±2,017	684,000± 7,410	0.6079±0.0077
536.....	5.19±.236	56.90±1.281	14.17±.148	.798±.0101	161,700±2,904	760,600±12,240	.7188±.0119
548.....	6.04±.270	53.75±1.207	15.71±.121	.829±.0115	123,800±2,380	570,400±10,140	.5101±.0088
554.....	6.33±.263	65.85±1.674	15.05±.205	.790±.0108	164,000±2,782	691,400±13,340	.7177±.0119
555.....	5.16±.216	72.10±1.349	16.54±.175	.762±.0081	151,100±2,262	581,900±10,580	.7328±.0096
559.....	6.78±.290	60.70±1.281	15.78±.148	.813±.0074	135,800±2,249	581,000± 8,600	.6060±.0085
567.....	6.68±.308	54.75±1.200	14.75±.136	.806±.0074	146,000±2,925	652,500± 9,020	.6296±.0069
Averages	6.48±.100	59.62±.494	15.15±.058	.812±.003	147,600± 955	646,000± 3,920	.6461±.0037

YEARLING EWES

(SAME PASTURES AND FEED AS ABOVE)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
506.....	6.29±0.243	82.75±1.551	16.34±0.121	0.661±0.0081	171,600±2,616	658,500± 8,350	0.7767±0.0143
530 ¹	8.97±.283	138.30±2.428	22.20±.216	.509±.0080	159,900±2,214	520,700± 8,340	.6621±.0093
508.....	9.17±.344	58.80±1.281	14.57±.128	.798±.0094	158,600±2,876	680,000± 9,750	.6287±.0074
517.....	6.90±.229	74.30±1.403	15.04±.155	.748±.0096	166,700±2,536	630,800± 7,404	.7645±.0105
524.....	6.30±.243	71.43±1.227	15.70±.133	.741±.0070	165,900±2,443	642,600± 9,380	.8559±.0100
528.....	7.32±.283	79.60±1.619	17.28±.155	.760±.0088	146,200±2,406	521,800± 9,530	.5713±.0106
529.....	6.02±.296	63.25±1.349	15.46±.175	.798±.0114	169,300±2,684	649,100±13,230	.7275±.0091
541.....	8.09±.374	54.50±.944	12.49±.094	.913±.0108	197,500±2,555	830,000± 9,54	.7547±.0099
552.....	7.36±.276	61.70±1.214	15.07±.116	.704±.0073	153,300±2,554	724,800±11,270	.5969±.0095
553.....	7.80±.283	50.40±1.012	12.98±.140	.869±.0108	170,300±2,424	804,900±12,840	.7208±.0112
556.....	8.01±.263	77.00±1.158	16.08±.125	.694±.0067	170,300±2,254	653,000± 7,140	.7673±.0107
Averages (10)	7.33±.087	67.37±.407	15.19±.043	.769±.0029	167,000± 804	679,500± 3,170	.7164±.0032

¹530 omitted from the averages. Her wool was strictly one-half blood.

TABLE 2.—*Tensile strength and elasticity of wool from yearling wethers and ewes, 1917—Continued*

YEARLING RANGE WETHERS

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>Mm.</i>	<i>Dgm.</i>	μ				
561-----	6.22±0.206	52.30±1.214	13.03±0.135	0.858±0.0148	170,900±2,923	850,900±16,400	0.9442±0.0145
565-----	4.98±.142	49.10±.068	12.92±.121	.938±.0141	170,800±2,673	780,000±13,610	.8207±.0131
569-----	7.00±.162	53.00±.853	13.30±.137	.900±.0090	175,000±3,274	751,000±14,530	.7853±.0151
Averages	6.07±.0991	51.47±.542	13.08±.076	.899±.0076	175,200±1,713	794,000± 8,600	.8521±.0082

YEARLING RANGE EWE

562-----	7.00±0.216	53.30±0.809	13.51±0.108	1.153±0.0148	168,200±2,945	565,800±7,840	0.8589±0.0098
Averages (4)---	6.30±.092	51.92±.454	13.19±.063	.962±.0068	173,500±1,481	736,900±6,740	.8538±.0066

TABLE 3.—*Tensile strength and elasticity of wool from aged ewes, 1918*

LOT 1

(EWES GRAZED ON IRRIGATED FENCED PASTURE; GOOD FEED IN WINTER; SHIELTER)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>Mm.</i>	<i>Dgm.</i>	μ				
529-----	6.92±0.189	55.36±1.012	14.64±0.121	1.012±0.0148	128,700±1,963	562,400± 8,090	0.6582±0.0108
530-----	8.52±.256	104.24±2.226	19.53±.209	.546±.0094	153,900±2,159	596,800±12,230	.7397±.0132
541-----	8.54±.249	42.40±.742	12.08±.121	1.213±.0256	168,400±3,105	775,700±16,860	.8504±.0170
578-----	9.81±.236	45.15±1.012	12.78±.148	1.362±.0290	159,700±3,100	576,500±11,060	.7419±.0146
221-572--	10.82±.249	73.94±1.583	15.68±.142	.884±.0135	177,200±3,283	557,900±11,120	.8615±.0174
392-696--	9.02±.216	58.03±1.079	15.00±.142	.988±.0121	150,900±3,195	563,800±12,190	.7488±.0193
393-961--	8.67±.256	50.26±.809	14.07±.121	.990±.0168	148,100±2,960	625,800±13,350	.7371±.0130
406-980--	7.04±.256	40.39±.877	12.78±.128	1.233±.0283	141,800±3,197	635,800±14,110	.7843±.0146
407-959--	9.16±.297	51.79±1.281	13.47±.169	1.153±.0229	160,300±1,966	590,200±11,110	.7929±.0095
508-678--	8.53±.196	52.29±.877	13.44±.121	.940±.0155	166,100±2,666	736,800±16,580	.7820±.0120
655-656--	9.72±.236	54.76±.944	13.89±.135	.938±.0215	163,100±3,130	695,900±16,590	.7847±.0152
Averages (10)---	8.82±.076	52.44±.332	13.78±.043	1.070±.0066	156,400± 916	632,100± 4,240	.7742±.0046

LOT 2

(SEMI-RANGE EWES)

517-----	9.12±0.222	62.19±1.147	15.94±0.167	0.925±0.0134	158,600±2,495	578,700±12,180	0.7693±0.0206
552-----	7.70±.182	55.76±1.279	14.09±.142	.850±.0148	168,000±2,482	725,200±14,550	.7461±.0125
80-463--	9.90±.206	45.70±.526	12.08±.088	1.154±.0182	181,200±2,750	723,200±14,310	.8635±.0140
390-690--	8.68±.202	46.76±.722	13.23±.135	1.244±.0202	155,700±3,073	566,300±10,700	.7836±.0136
401-489--	8.81±.209	50.21±.944	13.86±.135	1.027±.0162	151,000±2,478	615,300± 9,990	.7390±.0101
476-477--	7.30±.179	42.02±.742	13.40±.142	1.121±.0259	139,000±2,547	646,000±14,290	.7059±.0135
Averages (6)---	8.58±.082	50.44±.380	13.77±.056	1.053±.0076	153,900±1,081	642,400± 5,230	.7674±.0059

¹530 omitted from mean because of coarse fiber. This ewe produced half-blood wool.

The results of these tests are given in Tables 1, 2, and 3. Stretch represents total stretch at the breaking point. Break is the stress at the breaking point in decigrams. Diameters are expressed in

microns. Average elongation is the average stretch produced by the successive increments of 5 dgm. of stress. Tensile strength =

Breaking stress in decigrams
Area of cross section in square millimeters. Young's modulus = $\frac{L \times S}{E \times A}$.

The length of fiber under test (L) is expressed in millimeters; stress (S), expressed in grams, refers to the increments of 0.5 gm. added at regular intervals: Average stretch or elongation (E), expressed in units of 2 mm., refers to the average stretch produced by the increments of 0.5 gm. before the elastic limit point is reached. Elastic limit on equal area = $\frac{\text{Elastic limit point in decigrams.}}{\text{Area of cross section in sq. mm.}}$

All samples tested in these studies were taken from the middle of the shoulder.

Average results indicate lower values for range ewes for stretch, break, and diameter, and higher values for range ewes for average elongation, tensile strength, Young's modulus, and elastic limit on equal area. Although the individual results are not consistent, the probable errors of some of the averages are small enough in proportion to the differences between averages to make the results seem significant.

Similar but more widely differing results were obtained with yearlings in 1917 as indicated in Table 2. In 1918 the results with aged ewes agreed only in part with those obtained with similar ewes in 1917.

Composite graphs representing diameter and elastic limit on equal area are given in Figures 1 and 2. The graphs representing elastic limit on equal area are considered typical also of those representing tensile strength and Young's modulus. The latter are therefore not included in this paper.

Considering the composite graphs, a rather uniform tendency is noted toward a smaller diameter of fiber from the aged ewes as well as from the yearling wethers and the yearling ewe kept under range or semirange conditions as contrasted with the corresponding measurements from ewes kept under more favorable conditions as to feed, shelter, and general care. The data on tensile strength, Young's modulus, and elastic limit on equal area are not definite in their significance since the differences in the results obtained on aged ewes in 1918 are but little more than the probable errors of the means.

The greater strength of the smaller fibers per unit of area of cross section is explainable on the basis of the structure, the medulla obviously contributing less strength than the cortex in proportion to the areas of their respective cross sections. This relation in structure accounts for the fact that the tensile strength, Young's modulus, and the elastic limits on equal area are not proportional to the diameter. These quantities are functions of the respective areas of cross section of the cortex and medulla and of their respective strength, rather than of the total area of the cross section of the fiber or of the diameter of the fiber.

No data were available to indicate the basis of selection of the ewes placed on the range in 1916, and it was therefore not established that these ewes were closely comparable with those kept in the home flock. In other words, the effect of feed and other external conditions on the diameter of wool fibers could not be determined from the data for these ewes. It seems, however, that the strength and

elasticity as related to the diameter of the fibers were somewhat similar among the individuals in the two lots.

It seemed desirable to obtain a more accurate check on individuality and to reduce the number of variable factors in the experiment. Wethers were therefore selected for further study with the idea of eliminating such factors as oestrus, pregnancy, lambing, and milk production, and with the further idea of using these animals as subjects through a sufficient period of years to determine the effect of different rations on a series of fleeces produced by the same wether. In this way a check was obtained on the individuality of the wether.

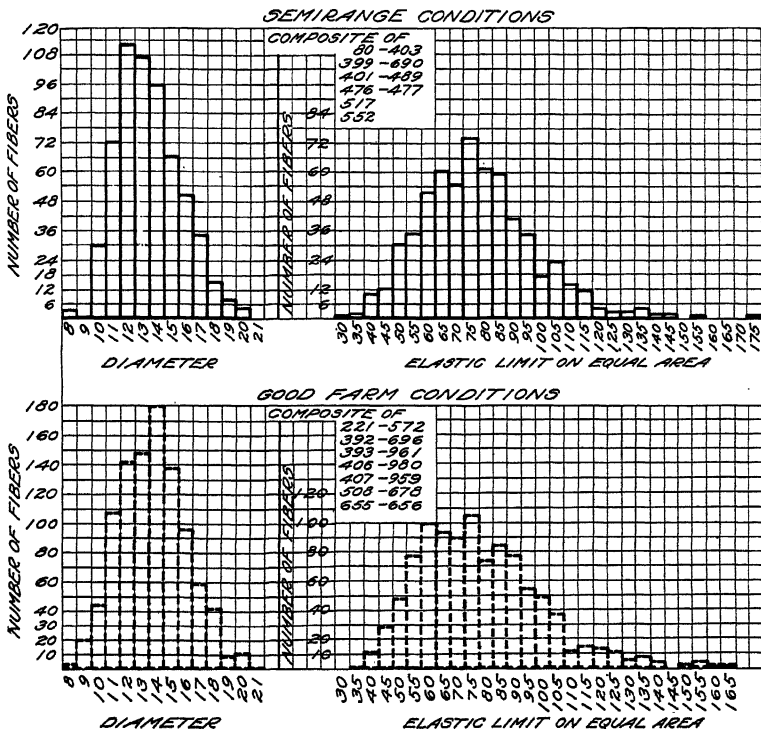


FIG. 2.—Composite graphs showing diameters and elastic limit on equal area of wool fibers from aged ewes kept under semirange conditions and under good farm conditions in 1918

The feeding period ranged from five to six months each year. During the remainder of the year the wethers were run together.

During the first year of the test one lot of wethers was fed an average daily ration of 4 pounds of alfalfa hay and 0.6 pound of a mixture of oats 3 parts and oil meal 1 part. The second lot was fed 4 pounds of mixed hay consisting of timothy 3 parts to medium red clover 1 part.

In 1920 and 1921 one lot was fed an average daily ration of 4 pounds of alfalfa of good quality. The other lot was fed oat straw which contained no grain and a very limited quantity of chaff and 4 ounces per head of cottonseed cake containing 43 per cent protein.

The results of the tests of wool fibers are given in Tables 4, 5, and 6 and in Figures 3, 4, 5, 6, and 7.

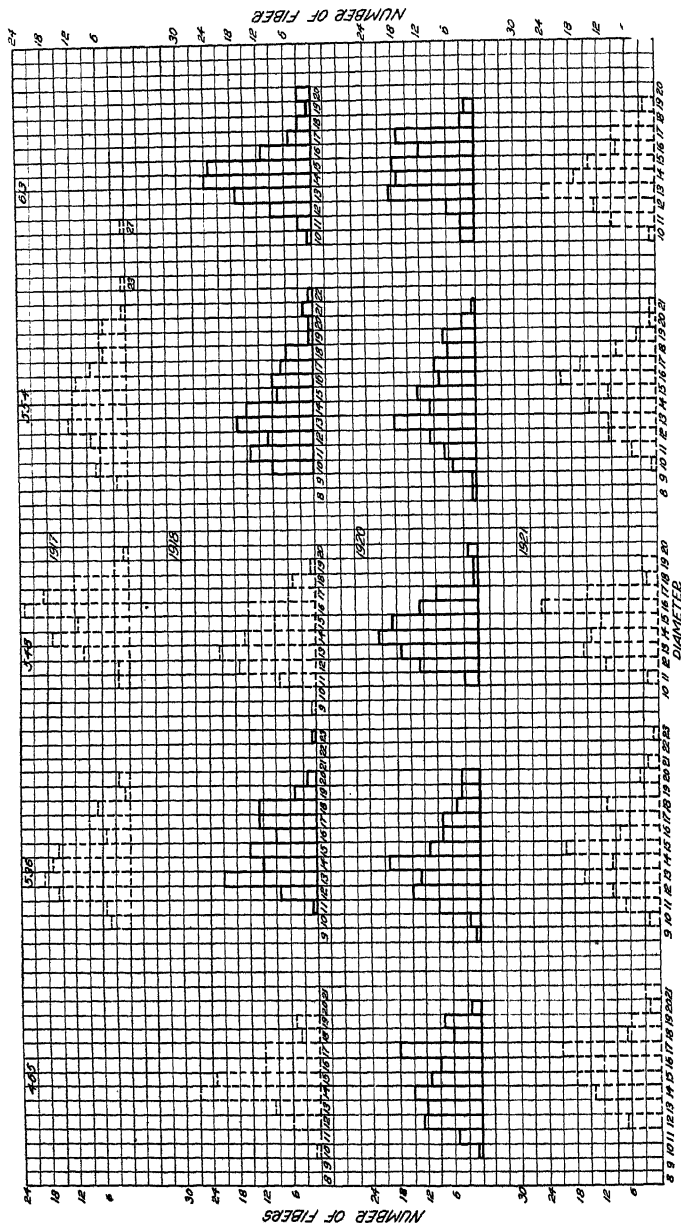


Fig. 3.—Diameters of wool fibers from wethers ranging in age from 1 to 5 years. Broken lines, liberal feed; solid lines, limited feed

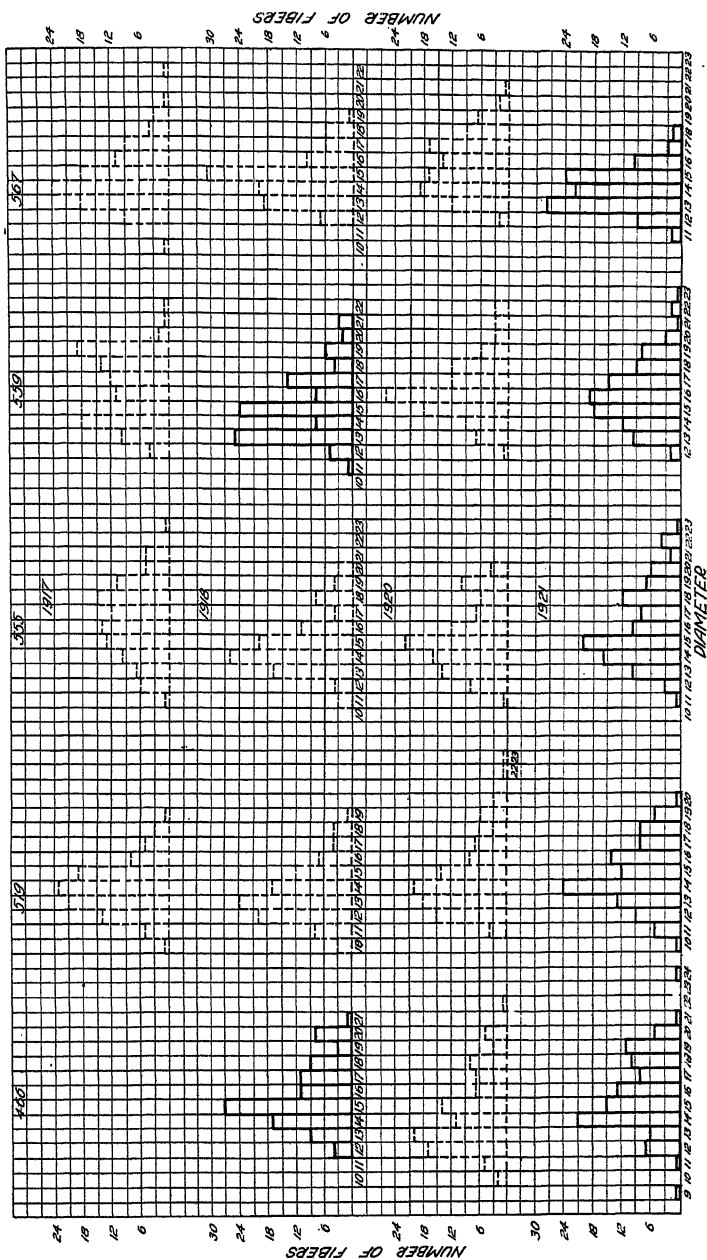


FIG. 4.—Diameters of wool fibers from wethers ranging in age from 1 to 5 years. Broken lines, liberal feed; solid lines, limited feed

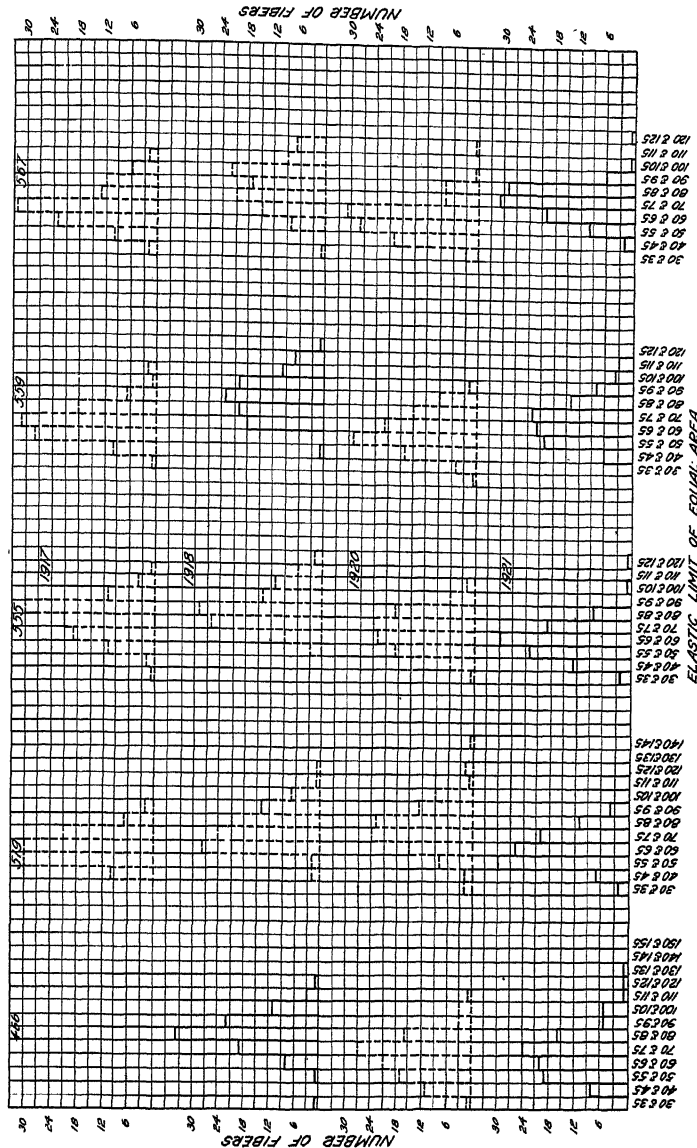


FIG. 6.—Elastic limit on equal area of wool fibers from wethers ranging in age from 1 to 5 years. Broken lines, liberal feed; solid lines, limited feed

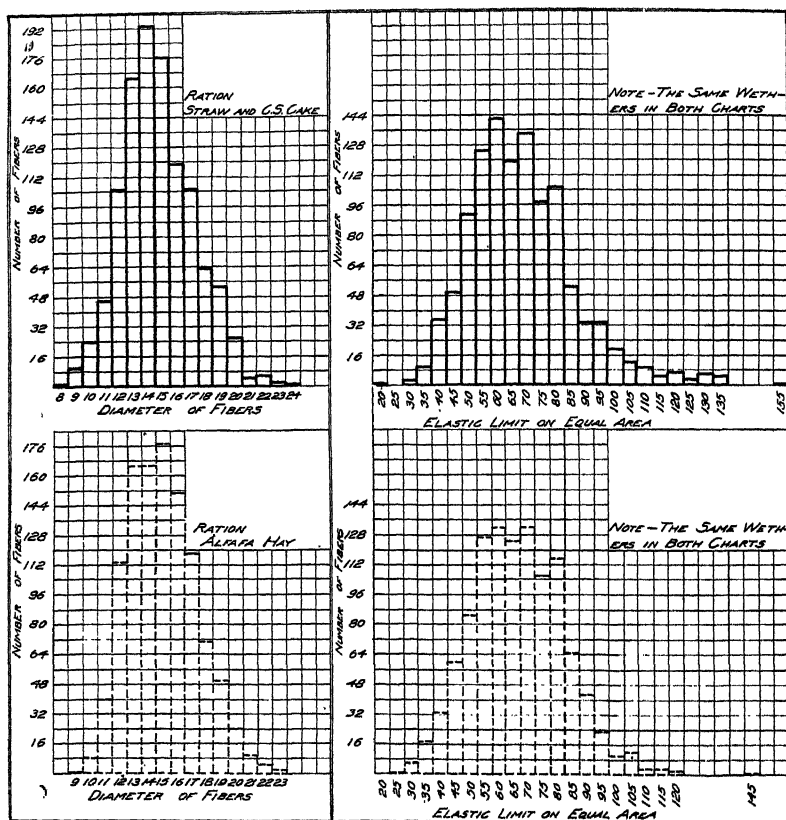


FIG. 7.—Diameters and elastic limit on equal area of wool fibers from 11 wethers, 1920 and 1921. One group of these wethers was fed straw and cottonseed cake during the winter of 1920 and alfalfa hay during the winter of 1921. The other group was fed these rations in the reverse order during the respective years. The same wethers are represented in all graphs, thus eliminating the variation which would result from the use of different sets of individuals in the two lots. Solid lines indicate limited feed, broken lines, liberal feed.

The graphs in this figure are composites of those in Figures 3, 4, 5, and 6 for the years 1920 and 1921, in which each wether was represented by a separate graph. By eliminating variation due to testing limited numbers of fibers from each sample, great uniformity of results is obtained even though the rations differed markedly in feeding value.

TABLE 4.—Tensile strength and elasticity of wool, 1918

LOT 1

(RAMBOUILLET WETHERS; RATION DURING THE WINTER FEEDING PERIOD, ALFALFA HAY, OATS, AND LINSEED-OIL MEAL)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>mm.</i>	<i>Dgm.</i>	<i>μ</i>				
465-----	10.24±0.243	59.34±1.214	15.10±0.115	1.161±0.0269	146,400±2,219	545,200±9,370	0.6751±0.0099
519-----	8.43±	229 50.74±1.214	13.61±	1.239±	0.0209 153,100±1,965	529,800±7,550	.7614±.0098
548-----	8.35±	236 49.89±	14.06±	1.126±	0.0188 144,300±2,041	555,500±11,010	.7344±.0118
555-----	7.52±	216 57.06±1.012	14.95±	.925±	0.0115 145,100±2,118	574,000±8,240	.8347±.0112
567-----	8.67±	236 58.25±1.079	14.65±	.999±	0.0134 152,900±2,119	553,800±6,840	.8064±.0112
Aver- ages--	8.64±.104	55.06±.491	14.47±.055	1.090±.0085	148,360±936	551,700±3,900	.7624±.0048

LOT 2

(RAMBOUILLET WETHERS; RATION DURING THE WINTER FEEDING PERIOD, MIXED HAY, I. E., TIMOTHY AND CLOVER)

466-----	9.05±0.229	69.66±1.416	15.71±0.142	0.858±0.0114	158,600±1,896	563,300±6,480	0.8424±0.0094
536-----	8.22±	229 57.31±1.416	15.38±	1.008±	0.0195 136,400±2,085	520,600±8,530	.6816±.0086
554-----	7.65±	209 55.25±1.281	13.92±	1.028±	0.0203 161,800±2,532	643,400±12,830	.8904±.0137
559-----	9.04±	256 63.29±1.281	15.24±	.911±	0.0121 154,100±2,196	571,200±8,780	.8265±.0102
613-----	10.15±	217 61.20±1.179	14.48±	.918±	0.0141 173,900±2,460	610,500±9,660	.8201±.0107
Aver- ages--	8.82±.102	61.34±.589	14.95±.068	.945±.0071	157,000±1,005	581,800±4,240	.8122±.0048

TABLE 5.—Tensile strength and elasticity of wool, 1920

LOT 1

(RAMBOUILLET WETHERS; RATION DURING THE WINTER FEEDING PERIOD, ALFALFA HAY)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>mm.</i>	<i>Dgm.</i>	<i>μ</i>				
466-----	5.62±0.276	57.32±1.281	14.63±0.179	1.081±0.0162	148,460±2,191	581,100±8,350	0.6694±0.01018
519-----	8.89±	263 59.94±1.214	14.72±	1.134±	0.0135 159,160±2,343	562,300±9,510	.7795±.01254
555-----	7.10±	297 60.66±1.214	15.45±	.864±	0.0115 146,440±2,144	585,100±8,040	.6771±.00951
559-----	6.27±	275 72.56±1.375	16.37±	.900±	0.0084 149,880±2,207	493,700±7,060	.6807±.00910
567-----	10.23±	303 66.14±1.012	15.73±	1.001±	0.0141 156,390±2,361	474,200±12,710	.6851±.00910
Aver- age--	7.62±.127	63.32±.548	15.38±.069	.996±.0058	152,070±1,007	539,300±4,220	.6583±.0046

LOT 2

(RAMBOUILLET WETHERS; RATION DURING THE WINTER FEEDING PERIOD, STRAW AND COTTONSEED CAKE)

465	6.78±0.238	55.59±1.099	15.06±0.167	1.129±0.0169	145,280±3,048	495,000±12,080	0.6209±0.01194	
492	9.04±	270 49.17±	850 13.35±	1.144	1.320±	0.0164 176,190±2,816	569,400±10,420	.7704±.01389
536	8.07±	249 59.68±1.214	14.33±	.169	.894±	0.0135 165,310±2,738	650,100±11,000	.7520±.01268
548	7.35±	256 59.21±1.402	14.79±	.126	1.012±	0.0157 157,800±2,553	566,000±7,240	.6482±.01031
554	8.98±	297 60.53±1.584	14.29±	.189	.988±	0.0209 161,300±2,560	597,300±10,870	.7002±.01409
613	6.22±	290 58.95±1.160	14.73±	.133	1.013±	0.0209 155,630±3,409	557,700±11,190	.6617±.01240
Average	7.74±.109	57.19±.506	14.42±.064	1.059±.0072	160,200±1,171	572,600±4,320	.6939±.00515	

TABLE 6.—Tensile strength and elasticity of wool, 1921

LOT 1

(RAMBOUILLET WETHERS; RATION DURING THE WINTER FEEDING PERIOD, STRAW AND COTTONSEED CAKE)

Sheep No.	Stretch	Break	Diameter	Average elongation	Tensile strength	Young's modulus	Elastic limit on equal area
	<i>mm.</i>	<i>Dgm.</i>	<i>μ</i>				
466.....	9.66±0.283	70.60±1.821	15.89±0.175	1.006±0.0243	154,800±2,515	159,600±7,160	0.6813±0.0117
519.....	7.94±.229	52.80±1.079	14.79±.148	1.464±.0378	137,280±1,954	385,800±5,890	.6316±.0086
555.....	8.86±.250	65.45±1.686	16.27±.182	1.152±.0189	136,820±2,116	397,500±5,200	.6173±.0100
559.....	9.20±.268	69.10±1.420	16.21±.140	1.028±.0174	149,420±1,830	152,900±7,010	.6906±.0107
567.....	11.10±.310	63.30±1.079	14.19±.096	1.148±.0296	178,120±2,482	510,100±6,180	.7235±.0082
730 ¹	9.66±.242	54.55±1.360	13.86±.162	1.316±.0270	160,120±2,674	482,500±7,190	.6459±.0104
730 ²	10.14±.310	57.40±1.110	14.41±.121	1.320±.0240	155,170±2,444	446,100±6,240	.6710±.0101
Average..	9.50±.103	61.89±.527	15.09±.056	1.212±.0100	153,110± 872	447,800±2,440	.6659±.0098

LOT 2

(RAMBOUILLET WETHERS; RATION DURING THE WINTER FEEDING PERIOD, ALFALFA HAY)

465.....	9.94±0.270	68.05±1.551	15.79±0.142	1.178±0.0187	153,600±2,335	411,000±5,710	0.6766±0.0087
492.....	12.66±.283	61.38±1.147	13.74±.115	1.408±.0311	168,070±2,599	471,800±9,650	.6580±.0105
536 ³	9.70±.270	61.75±1.416	14.90±.182	1.229±.0214	158,386±2,935	447,000±7,360	.6803±.0100
548 ⁴	9.72±.256	54.55±1.012	14.93±.128	1.382±.0322	141,960±2,031	395,500±5,770	.5869±.0085
554.....	11.04±.324	69.15±1.659	15.05±.155	1.166±.0243	167,410±2,457	447,600±5,780	.7111±.0088
613.....	11.62±.270	64.04±1.281	13.92±.128	1.098±.0202	182,140±2,191	554,500±6,770	.7331±.0088
759 ⁵	9.48±.268	61.15±1.110	14.26±.137	1.060±.0140	171,300±2,298	556,700±7,920	.6862±.0100
Average..	10.59±.105	62.87±.503	14.66±.054	1.217±.0090	163,270± 915	469,200±2,690	.6760±.0035

¹ Fifteen fibers broke while being adjusted in the machine.² One fiber broke while being adjusted in the machine.³ One fiber broke while being adjusted in the machine.⁴ Four fibers broke while being adjusted in the machine.⁵ Five fibers broke while being adjusted in the machine.

An analysis of the results therein shown, reveals no variation in stretch, break, diameter, average elongation, tensile strength, Young's modulus, or elastic limit on equal area which can be ascribed to differences in the feed during the test period. The small differences in results obtained on any individual from year to year appear to be due to errors in sampling and to variability of the limited numbers of fibers tested; in other words, to the normal experimental error. The charts showing the distribution of all fibers tested from a given group of wethers under different feed conditions show clearly how uniform the results were. Notwithstanding the rather wide variation in feed during the test period, the secretion of the wool fibers took place in a uniform manner in so far as size, strength, and elasticity of the fibers were concerned, and apparently the relations of the medulla and the cortex of the fibers remained the same under the two conditions.

The condition or degree of fatness of the wethers was clean cut at the end of the test-feeding period, those fed the more liberal ration uniformly carrying more fat. Those fed the limited rations during the last two years lost in weight, one wether actually losing at the rate of over one-third pound per day. Nor was there any question regarding the "condition" of the wool. The fleeces of the wethers in the lots fed straw and cottonseed cake were dry, harsh, and lifeless to the touch as compared to the fleeces of the wethers fed a liberal ration of alfalfa hay, which were in every case quite satisfactory in "condition."

The length of staple and weight of fleece were also considered in this connection. These are given in Table 7 in which they are arranged both chronologically without regard to the method of feeding and according to the method of feeding without regard to sequence in time.

TABLE 7.—*Weight of fleece and length of staple, 1920 and 1921*

ARRANGED CHRONOLOGICALLY

Wether No.	Weight of fleece in pounds		Length of staple in inches	
	1920	1921	1920	1921
465.....	12.8	14.6	2.34	2.17
466.....	13.2	11.1	2.69	2.40
492.....	10.9	11.7	2.81	2.72
519.....	14.6	12.7	2.37	2.27
536.....	12.9	13.2	2.98	2.71
548.....	13.0	14.1	2.10	2.16
554.....	13.7	15.8	2.76	2.63
555.....	16.6	12.5	3.04	2.47
559.....	14.6	12.4	3.36	2.98
567.....	18.2	16.1	2.76	2.55
613.....	¹ 12.7	² 13.9	¹ 2.70	² 2.70

ARRANGED ACCORDING TO RATION FED

Wether No.	Hay		Straw and cake	
	Hay	Straw and cake	Hay	Straw and cake
465.....	14.6	12.8	2.17	2.34
466.....	13.2	11.1	2.69	2.40
492.....	11.7	10.9	2.72	2.81
519.....	14.6	12.7	2.37	2.27
536.....	13.2	12.9	2.71	2.98
548.....	14.1	13.0	2.16	2.10
554.....	15.8	13.7	2.63	2.76
555.....	16.6	12.5	3.04	2.47
559.....	14.6	12.4	3.36	2.98
567.....	18.2	16.1	2.76	2.55
613.....	13.9	12.7	2.70	2.70

¹ Second fleece.² Third fleece.

Arranged chronologically, the weights of the fleeces show complete absence of uniformity in tendency, but arranged according to the ration fed, the fleeces grown during the period in part of which hay was fed were uniformly heavier than those produced during the year represented by the test period on straw and cake.

With two exceptions the length of staple shows a uniform tendency to decrease with the advancing age of the wethers. One of these exceptions was that of number 613 which was 2 and 3 years old during the respective years of the test, whereas the other wethers were 3 and 4 or 4 and 5 years old during the same period. The second exception was that of a wether which was fed straw and cake during a part of the first year and hay during a part of the second year of the test. There is no tendency toward a decrease in length of staple in the third fleece as compared with the second fleece as is indicated by unpublished data.

Considering the data on length of staple as arranged in the second part of the table, there is complete lack of uniformity in tendency. Interpreting the two parts of the table, age is a greater factor in

determining length of staple than rather marked differences in the level of feeding continued over a period of five to six months. In other words, the length of staple will be modified almost imperceptibly by the level of feeding during the average feeding period on the ranges in Montana unless extreme differences are considered. The rate of secretion of the wool fiber during a short feeding period in winter, while subject to influence by feeding, is modified significantly only with some difficulty and under rather extreme conditions. Careful analysis of the data reveals a slight difference between the lots which is probably due to a more rapid decrease in length of staple if the ration of hay was fed the first year and straw and cake the second year, than if the rations were fed in the reverse order. In other words, while the feed as tested in this experiment seemed to influence the length of staple, this influence was purely secondary to that of age and therefore not capable of exact measurement with limited numbers of sheep.

There are opposed tendencies in length of staple and weight of fleece in this case, and there is no change in diameter of fiber or apparently in the quantitative relations of such structural parts of the fiber as influence its strength or elasticity. This is interpreted as indicating that while the physical properties of the wool fiber itself are not easily changed either quantitatively or qualitatively, the "yolk" or "grease" and other factors that go to make up shrinkage are easily subject to influence by the level of feeding even during a comparatively short period.

A very interesting case observed during this trial was that of wether No. 555 whose weight decreased over one-third of a pound per day during the feeding period of 174 days in 1921. The weight of his fleece decreased 4.1 pounds and the length of staple decreased 0.57 inch, but the diameter and the strength and elasticity of the wool fibers from his fleece were not influenced perceptibly by the level of feeding, notwithstanding the fact that he was so poorly nourished that he succumbed to an invasion of pus organisms even after he had been on pasture for some time following shearing and the close of the test period.

SUMMARY

The results of the investigation here reported indicate that in sheep of fine-wool breeding, the organs which are concerned with the secretion of the wool fiber are not easily subject to such influences as changes in the level of feeding, especially for periods of five to six months, if the sheep remain normal in health; that the quality of the wool fiber is affected not at all, and that the quantity of fiber may be modified only slightly, as long as the sheep remain in normal health; that age of wethers up to 5 years affects the diameter of wool fibers very slightly if at all; that advancing age after the third or fourth year causes a decrease in length of staple; that failures in selection of sheep for the production of adequate length of staple or amount of actual clean wool can not be compensated by better feeding during a short period in winter; that the level of winter feeding of sheep should be determined on the basis of the needs for other purposes such as creating or maintaining strength and condition for withstanding the rigors of winter, growth of body rather than of wool, production of strong lambs and of ample flow of milk at lambing

and after lambing; that if these points are properly observed the growth of wool will be provided for on a basis commensurate with its importance in the particular case; that any additional weight of fleece produced by more liberal feeding in winter consists of the factors making up shrinkage very largely and of actual wool fibers only slightly.

Although the fleeces produced during the years in which the wethers were fed at the lower levels lacked in "condition," it is still a question whether the spinning quality of the wool after scouring would be affected thereby; also, if adversely affected, whether the apparent lack of natural oily secretions could be compensated by the artificial application of oil.

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A STRAIN OF YELLOW JERSEY SWEET POTATO RESISTANT TO SURFACE ROT (*FUSARIUM OXYSPORUM* W. & C.)¹

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The loss of sweet potatoes in storage caused by surface rot is often large, especially in the Jersey varieties.^{2, 3} Harter and Weimer² have reported losses as great as 25 per cent. Data are available (Table 1) which show that as high as 38 per cent of Yellow Jersey roots have become infected with surface rot under certain storage conditions.

TABLE 1.—*Resistance to surface rot of the selected strain of Yellow Jersey sweet potatoes as compared with the unselected stock*

Seed from which roots were taken	Number of crates used	Number of roots used	Number of roots infected	Percentage infected
Selected.....	6	777	50	0.6
Unselected.....	4	609	233	38.0

While examining Yellow Jersey roots in storage in 1923 and 1924 the writer observed that two types were present, one of which was free from surface rot. These roots were distinguished from the others, not only by the absence of the disease but by other characters. The two strains may be described as follows: The resistant strain is fusiform in shape, dark russet in color, and the skin has a tendency to crack at the root end. The cracking is very slight and does not affect the value of the strain. In some roots it is entirely absent. The susceptible strain is globose and light russet. The coloring is really dependent upon or correlated with the degree of russetting, with some variation in both strains. The difference is sufficiently marked to distinguish the two strains readily.

The origin of the strains is not known. There has been no opportunity for the roots used by the writer to become mixed since they came into his possession. Probably the strains had their origin at an earlier date, for they have been observed in commercial stocks. It is possible that they may have come from seed in their original home in the tropics, and always have been mixed since they were brought to this country, or they may have arisen from bud variations here. Harter⁴ discovered three red roots and three yellow roots growing from the same vine of the Haiti variety of sweet potato in the fall of 1923. These two strains have reproduced true to type each season since.

¹ Received for publication July 26, 1926; issued December, 1926.

² HARTER, L. L., and WEIMER, J. L. THE SURFACE ROT OF SWEET POTATOES. *Phytopathology* 9: 465-469, illus. 1919.

³ POOL, R. F. SWEET POTATO DISEASES IN NEW JERSEY. *N. J. Agr. Expt. Sta. Circ.* 141, 31 p., illus. 1922.

⁴ HARTER, L. L. BUD SPORTS IN SWEET POTATO. *Jour. Agr. Research* 33: 523-525, illus. 1926.

A small number of roots resistant to surface rot were selected in the spring of 1924 and a planting was made from them along with a planting from unselected roots. The vines from the two plantings could not be distinguished from each other. The roots, however, reproduced true to type.

The plants from the resistant roots yielded 6 bushels of potatoes. These were stored in crates on October 10 in the same room and under the same conditions as those from the unselected seed (cured for 10 days at a temperature ranging from 21° to 24° C., stored at a temperature ranging from 10° to 15° and a relative humidity ranging from 55 to 75 per cent).

An inspection was made on March 11, the following spring, of all the selected stock and four crates of the unselected. The results are recorded in Table 1. The roots of the resistant strain showed only three-fifths of 1 per cent infection, whereas those from the unselected seed showed 38 per cent. This contrast becomes more striking when it is considered that there were many roots of the resistant strain mixed with those of the unselected stock.

In order to compare the resistant strain more directly with the susceptible, a selection of the latter was made in the spring of 1925, and a planting made from the two. Both reproduced true to type.

Sixty crates of the resistant and 48 of the susceptible strain were divided into four equal lots in each case and one lot of each strain was cured for 10 days under each of four different conditions of curing: (1) Temperature 21° to 24° C., relative humidity 70 to 80 per cent; (2) temperature 24° to 27°, relative humidity 45 to 60 per cent; (3) temperature 27° to 29°, relative humidity 45 to 55 per cent; and (4) temperature 29° to 32°, relative humidity 40 to 50 per cent. The potatoes of each strain under each curing condition were then subdivided into four equal lots, one of which was stored at each of four different humidities (62 to 68, 73 to 76, 77 to 84, and 81 to 87 per cent), the temperature being practically the same in each case. Each strain was thus subjected to 16 different combinations of conditions as to curing and storage humidity. The storage period extended from October 16, 1925, to March 10, 1926.

The results are summarized in Tables 2 and 3 in such a way that one can compare directly the effect on the development of surface rot of curing on the one hand and of humidity of storage on the other. The total number of infections under all conditions is also given.

The variation in the percentage of infection of the resistant strain under the conditions given in the tables ranged from 3 to 13, that of the susceptible from 35 to 57. The highest percentage of infection in the resistant strain obtained under any of the 16 combinations of conditions to which the roots were exposed was 21 (curing temperature 29° to 32° C., relative humidity 62 to 68 per cent). The percentage of infection of the susceptible strain under the same conditions was 55. The highest percentage of infection of the susceptible strain was 64 (curing temperature, 29° to 32°, relative humidity 77 to 84 per cent). The percentage of infection of the resistant strain under the same conditions was 6. The lowest percentage of infection obtained with the resistant strain was 1 (potatoes cured at 21° to 24° and stored at relative humidities of 81 to 87 per cent and potatoes cured at 24° to 27° and stored at relative humidities of 81 to 87 per cent). The percentage of infection of the susceptible

TABLE 2.—Resistance to surface rot of the susceptible and resistant strains of yellow Jersey sweet potato under different conditions of curing^a

Curing temperature, °C.	Curing relative humidity, per cent	Storage temperature, °C.	Storage relative humidity	Resistant strain			Susceptible strain		
				Number of roots used	Number infected	Percentage infected	Number of roots used	Number infected	Percentage infected
21 to 24....	70 to 80..	10 to 15	{The data recorded at each of the curing temperatures and humidities were obtained from potatoes stored at each of the storage humidities recorded in Table 3.	2,683	167	6	1,528	616	40
24 to 27....	45 to 60..	through-		2,191	79	4	1,509	529	35
27 to 29....	45 to 55..	out stor-		2,110	191	9	1,520	624	41
29 to 32....	40 to 50..	age period.		2,199	230	10	1,495	845	57
Total.....				9,185	667	7	6,052	2,614	43

^a Duration of curing and storage periods, 10 and 127 days, respectively.TABLE 3.—Resistance to surface rot of the susceptible and resistant strains of yellow Jersey sweet potato under different storage humidities^a

Storage relative humidity, per cent	Storage temperature, °C.	Curing temperature and relative humidity	Resistant strain			Susceptible strain		
			Number of roots used	Number infected	Percentage infected	Number of roots used	Number infected	Percentage infected
81 to 87....	10 to 15	{The data recorded at each storage humidity were obtained from potatoes cured at each of the curing temperatures and humidities recorded in Table 2.	2,274	60	3	1,397	515	37
77 to 84....	through-		2,642	192	7	1,469	556	38
73 to 76....	out stor-		2,562	194	8	1,594	740	46
62 to 68....	age period.		1,707	221	13	1,592	803	50
total.....			9,185	667	7	6,052	2,614	43

^a Duration of curing and storage periods, 10 and 127 days, respectively.

strain under the same conditions was 44 and 28, respectively. The lowest percentage of infection of the susceptible strain was 24 (curing temperature 24° to 27° and relative humidity 77 to 84 per cent and at curing temperature 27° to 29° and relative humidity 81 to 87). The percentage of infection of the resistant strain under the same conditions was 3 and 5, respectively. The total percentage of infection of the resistant strain under all conditions was 7, while that of the susceptible was 43.

It is not the purpose of this paper to discuss curing and humidity as factors in the development of surface rot, but merely to show that the resistant strain exhibits a marked resistance under a variety of conditions, conditions that are evidently favorable to surface rot.

The results here reported show that the dark strain (resistant strain) is markedly resistant to surface rot, although it is not entirely immune. This resistance is further evidenced by the fact that the lesions in the resistant strain are fewer in number and shallower than in the susceptible. There is also a tendency in the susceptible strain for the roots to shrivel about the lesions, which is almost entirely absent in the resistant. This tendency to shrivel is not to be re-

garded as a result of disease, however, but rather as a characteristic of the susceptible strain, for the roots shrivel at the tip to a marked degree even when they are not infected with surface rot. The resistant strain, on the other hand, shows very little tendency to shrivel even under very dry conditions.

It is proposed to call the resistant strain Improved Yellow Jersey for the following reasons: (1) Because of its resistance to surface rot and (2) because it withstands shriveling to a marked degree.



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SENSES OF THE COTTON BOLL WEEVIL—AN ATTEMPT TO EXPLAIN HOW PLANTS ATTRACT INSECTS BY SMELL¹

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INTRODUCTION

A thorough and comprehensive investigation of the senses and sense organs of the cotton boll weevil (*Anthonomus grandis* Boh.) will undoubtedly throw light on the means by which the cotton plant attracts this insect. Entomologists have considerable faith in the theory that there is some definite or indefinite relation, or possibly attraction, between insects and their host plants. In regard to some of the insect-pollinated plants it has been proved that the relation is a definite and mutual one, the insects and plants both being benefited; the insects receiving nectar or pollen in exchange for the unintentional labor of cross-pollination. In this case the insects are attracted either by sight or smell, but more probably by both. In other cases, however, the attraction is certainly not mutual, because only the insects profit by the relation. In such cases, how are the insects attracted—by sight or smell, or by some unknown sense? For short distances we believe that either sight or smell would suffice, but boll weevils are apparently able to locate cotton plants from long distances and do not seem to make mistakes in doing so. If smell is the chief or only factor to be considered, the most reasonable theory is to suppose that plants, like animals, emit odors, and that insects in searching for food, either for themselves or their progeny, are guided by them. It may also be assumed that the volatile chemical constituents of plants emit particular odors, and that the odor emitted from a plant is a combination of all the odors from these constituents, or possibly one odor may be so strong that it masks all the others. Upon this hypothesis, the odors from plants would vary according to the number, combination, and quantitative percentages of the various volatile constituents. Reasoning along this line, we may be able to explain why a few insects have only one host plant; why many have a preferred host plant, but will eat allied plants; and why others feed upon a large number of plants. If we had positive information on this subject, we might be able to devise practical methods for the control of the boll weevil and certain other insect pests by using attractive poisoned baits, or by placing the "attractant" in traps.

¹ Received for publication July 3, 1926; issued January, 1927.

The foregoing is only a brief theoretical statement of how plants attract insects by odors. At once the reader may rightly ask, "Is there any definite information to support this theory, and are insects really attracted by odors from a distance?" A mass of literature dealing indirectly with this subject has been published, but very little of it is actually applicable to this particular problem, chiefly because nearly all of the investigators have used certain chemicals, or other compounds, not necessarily plant constituents, and for that reason it is difficult to correlate the results. For lack of space only a few references will be cited. Verschaffelt's (57)² results are the most important along this particular line. He observed that cabbage butterflies seem to be attracted to various members of the Cruciferae, and found by tests that their larvae ate the leaves of 15 species, representing 14 genera of this family. Chemists had previously determined that all of these contain mustard oils; thereupon Verschaffelt devised experiments and proved that the larvae actually seek out various mustard oils.

There is no definite or reliable information to show that insects are attracted from long distances by plant odors, but members of the Biological Survey of this department have had success in attracting mountain lions, wildcats, and lynxes from a distance to traps baited with oil from the catnip plant, although this particular subject may have little or no bearing on that discussed in this paper. Nevertheless, we do have definite information about the attraction of insects from long distances by insect odors. Riley (50, p. 38-39) reports that a caged female of the *Ailanthus* silkworm moth of Japan was apparently scented from a long distance by a marked male of the same species, which had been carried one and a half miles one evening and then released; the next morning the two moths were together. Fabre (8, p. 189-216), Forel (9, p. 76), and Mayer (40) conducted many experiments to prove that male moths apparently by smell alone can easily locate their females from long distances.

No direct evidence has been presented to support the theory that cotton plants really attract boll weevils by means of odors or emanations, but Power and Chesnut (44, 45) have shown experimentally that living cotton plants do give off emanations containing two odorous substances, one of which has an extremely strong and persistent odor, and the present writer (38) in 1925 experimentally convinced himself that the Colorado potato beetle (*Leptinotarsa decemlineata* Say) is attracted partially, if not wholly, by the emanations from its host plants.

While conducting experiments at Tallulah, La., in July and August, 1922, to determine whether or not the practice of dusting cotton plants with calcium arsenate is injurious to honeybees, the writer was asked by B. R. Coad, in charge of the Delta Laboratory at Tallulah, to study the behavior of the cotton boll weevil in the field and laboratory. During the latter half of June, 1924, the writer was again at Tallulah, collecting more material and conducting further experiments on the boll weevil. The results obtained are herein recorded. Although only of a preliminary nature, they add considerable weight to the remainder of this report, which is of a morphological character.

² Reference is made by number (italic) to "Literature cited," p. 1139.

The writer has made a thorough study of the anatomy of the sense organs of the boll weevil, and has brought together the available information along this line in the hope that new light may be thrown on the entire subject.

ATTRACTIVENESS OF COTTON PLANTS TO BOLL WEEVILS

The boll weevil, compared with many other insects, is a weak flyer, but during the dispersal period in autumn it has been known to migrate more than 40 miles in a short time by successive short flights. At other periods it is comparatively quiet and seldom travels far, although in spring, when it emerges from hibernation, it may be a mile or more from the nearest cotton. It is, however, usually much nearer. Regardless of its distance from cotton plants, Hunter and Pierce (18, p. 41) inform us that it apparently makes no mistakes in finding its favorite food.

- The cotton boll weevil is known to eat normally several species of plants; but when forced to feed upon others to which it is not accustomed, it will partake of them only to a limited extent; however, practically all the plants which it will eat belong to one family, the Malvaceae. Coad (4, p. 3) and Smith (53, p. 5) state that the boll weevil normally feeds on the various species of the cultivated and wild cottons (*Gossypium hirsutum*, *G. herbaceum*, *G. barbadense*, *G. brasiliense*, *G. davidsoni*, etc.) and also on the so-called wild cotton (*Thurberia thespesioides*). Coad succeeded in rearing weevils on the buds of the shrubby althaea (*Hibiscus syriacus*), but had only partial success in rearing them on the buds of *Callirrhoe involucrata*, *C. pedata*, and *Sphaeralcea lindheimeri*.

CHEMOTROPIC EXPERIMENTS

Hunter and Hinds (17, p. 70-71) report that owing to the belief that it might be possible to destroy the weevil by attracting it to sweetened poisons, a number of experiments were performed in the laboratory and field. Various grades of sugar, molasses, and honey were used in comparison with the attractive influence of cotton leaves. None of the sweets seemed to exert any noticeable attraction, except the honey, and that only slightly; but the cotton leaves always attracted many weevils. Hunter and Pierce (18, p. 43) corroborate these findings.

Recently this theory has been revived and many tests conducted to determine whether it has any practical application. McGehee (26, p. 7, 15) does not believe that the molasses in the sweetened poison attracts the weevils, although this method compared favorably in its results with the dust method. Leiby and Harris (24, p. 9, 12, 18), who tested a proprietary sweetened poison, and home-mixed ones containing blackstrap molasses, state that their findings are inconclusive as far as results are concerned. Isely and Baerg (19, p. 16, 17, 23) used proprietary sweetened poisons and found that they appeared less effective than the dust method and involved much more labor.

EXPERIMENTS IN WHICH SWEET SUBSTANCES, WATER, COTTON LEAVES, AND COTTON SQUARES WERE USED

In order to find a substance, preferably a sweet one, which would attract boll weevils equally as well as do cotton squares, the experiments described below were performed at Tallulah, La., during July

and August, 1922. If such a substance were found, it could be mixed with poisons and applied in cotton fields to control the weevils; or traps baited with it might be installed in the fields for the purpose of catching the weevils. The writer was ably assisted by G. L. Smith, who performed the field tests and aided with those at the laboratory.

PRELIMINARY EXPERIMENTS

The first few preliminary experiments showed that weevils pay little or no attention to three brands of molasses (blackstrap, koo-koo, and karo), honey, saccharine (strong solution), and sugar sirup (saturated solution).

Fifty weevils, collected the day before being tested, were put in each of eight wire-screen cages (12 inches square by 1.5 inches deep) which were placed side by side on supports in the shade of a large cottonwood tree. Experience quickly showed that most of the weevils went to one corner or to one side of the cage and usually remained there, regardless of the substance placed in the cage; therefore, in order to force them to change their position, it was necessary before testing them to reverse the cages end for end. The seven liquids used were put on small pieces of absorbent cotton of equal size, and the pieces of cotton were then laid on small pieces of paper of equal size, one of which was placed at the center of each of seven cages. A cotton square, lying on a piece of paper of the same size, was also placed in the center of the eighth cage. Counts were made every minute up to 10 minutes, when the liquids and cotton squares were shifted from cage to cage, so that at the conclusion of the experiments each of the eight cages had contained each of the eight substances. A summary of these eight series of tests at the end of the 10 minutes showed the total number of weevils which had eaten of the substances to be as follows: Cotton square, 27; water, 8; honey, karo, and koo-koo, 7 each; sugar sirup and saccharine, 5 each; and blackstrap, 3.

The following morning about half of the weevils were dead. The foregoing experiments were repeated by using 25 of the remaining weevils, now hungry, in each cage. A summary of these tests at the end of 10 minutes showed the number of weevils which had eaten of the various substances to be as follows: Cotton square, 21; water, 11; honey and karo, 6 each; sugar sirup and koo-koo, 3 each; blackstrap, 1; and saccharine, 0.

The preceding experiments were repeated three times, 50 fresh weevils being used in each cage each time, although in these tests the liquids were dropped upon fresh cotton leaves which were placed at the centers of the cages. A summary of all three sets gives the following results: Cotton square, 172; water and sugar sirup, 58 each; karo, 53; honey, 44; koo-koo, 35; blackstrap, 21; and saccharine, 15.

Tests in which 30-minute periods were used instead of 10-minute ones did not materially change the above proportions.

TESTS IN FIELD CAGES

Experiments somewhat similar to the preceding, but performed on a larger scale, were carried on by releasing 100 fresh weevils in each of 7 field cages, 4 feet square by 4 feet deep. The cages were turned upside down and heavy muslin used as tops, which made more or less shade for the weevils. The various liquids to be tested

were dropped upon fresh cotton leaves, one of which was then placed at the center of each side and bottom of the cage. Cotton squares and leaves not bearing any liquid were similarly placed. The weevils were released at 10.30 a. m., and counts were made every half hour up to 5 p. m. A summary of two sets of experiments, showing the maximum number of weevils at one time on all the leaves or squares in a cage, is as follows: Cotton square, 28; koo-koo, 7; honey, 6; blackstrap, 5; karo, 4; leaves (control), 4; and sugar sirup, 1.

The field cages used in the preceding experiments were moved to a cotton field and each was placed over a cotton plant in the usual manner when such cages are employed. At 8.30 a. m., about 12 leaves at the top of a plant were treated with drops of either sugar sirup, honey, karo, koo-koo, blackstrap, or with ice-cream powder in a liquid form. The control plant was left untreated. At 9 o'clock, 100 fresh weevils were released in each cage, and thereafter up to 5 o'clock the number of weevils found on the treated plants and control were counted hourly. This set of weevils was used two days, the liquids being applied to the leaves again on the second day.

These experiments were repeated twice, fresh weevils being used each time. The second set of insects was used three days and the third set two days. In these five tests a sweetened proprietary calcium arsenate mixed with water was also applied to the leaves in addition to the substances enumerated above.

TABLE 1.—Maximum number of boll weevils found at one time on untreated plant and on other cotton plants variously treated

Test No.	Number of weevils on untreated plant		Number of weevils on plants treated with—													
			Sugar sirup		Honey		Karo		Koo-koo		Blackstrap		Ice-cream powder		Sweetened proprietary calcium arsenate	
			Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves
1	89	91	3	92	4	92	1	86	1	79	1	88	5			
2	84	83	5	85	7	80	2	81	2	66	1	86	5			
3	85	88	3	85	3	91	2	72	2	60	0	85	4	62	0	
4	96	94	3	82	3	95	2	82	3	96	0	95	4	90	0	
5	83	78	4	73	2	85	3	69	3	74	2	93	3	63	0	
6	91	86	3	91	3	95	3	88	1	85	2	95	3	95	0	
7	70	84	3	73	2	85	1	82	2	73	0	85	2	86	0	
Average	85	86	3	83	3	89	2	80	2	76	1	90	4	79	0	

Tables 1 and 2 show the detailed results of these seven tests. It is seen that the plants treated with karo and ice-cream powder bore a few more weevils than did the control; those treated with koo-koo, blackstrap, and sweetened calcium arsenate bore a few less; and those treated with sugar sirup and honey bore practically the same number as did the control (Table 1). Since these differences are

so small, they may be attributed to probable errors. The maximum number of weevils found at one time on the treated leaves shows that the liquids used had little or no attractiveness, but the black-strap and sweetened calcium arsenate seem to have been slightly repellent (Table 1). Relative to the total number of weevils found on the treated leaves during the full period of the experiments, Table 2 shows that honey and ice-cream powder might have had a slight attractiveness, although so slight that they could have been of no practical importance.

TABLE 2.—*Number of boll weevils found on treated leaves of cotton plants during the full period of experiments*

Test No.	Number of weevils on leaves treated with—						
	Sugar sirup	Honey	Karo	Koo-koo	Black-strap	Ice-cream powder	Sweetened proprietary calcium arsenate
1.....	7	15	2	2	2	13	-----
2.....	11	15	5	4	1	13	-----
3.....	11	18	8	6	0	13	0
4.....	6	12	3	6	0	7	0
5.....	12	10	6	8	4	18	0
6.....	11	7	8	1	6	15	0
7.....	8	6	3	5	0	11	0
Average.....	9	12	5	5	2	13	0

CONCLUSIONS

The results obtained in this investigation indicate that the various substances used attract boll weevils very little or not at all, and none of them is of any practical value as a means of controlling these insects.

OLFACTORY EXPERIMENTS

The following observations were made by the writer in 1922 while trying to find a sweet substance that would attract boll weevils. As already stated, nearly all of the weevils tested in the cages (12 inches square) for some unknown reason went to the west side, and there remained regardless of the substances in the cages. Just as soon as the cages were reversed end for end, the weevils immediately walked from the east to the west side, and in so doing many of them passed within one-quarter, one-half, or three-quarters of an inch of the sweet substance on the absorbent cotton or on the cotton leaves, yet in hardly an instance did they turn from their straight course to go to it. They behaved differently, however, toward the cotton squares. Many of them went directly to the squares from a distance of 4 or 5 inches; and often a weevil turned at right angles while passing a square in order to reach it. If the honey and five brands of sirup used had acted as attractants, the weevils certainly would have behaved similarly toward them; although in regard to all of these responses, sight instead of smell might have been the sense used.

In collaboration with this bureau, F. B. Power and V. K. Chesnut, of the Bureau of Chemistry, undertook to determine the volatile constituents of the cotton plant in order that these substances might be used to attract boll weevils. This work was begun at Tallulah,

La., in the summer of 1923 by distilling large quantities of cotton plants, and owing to the complex nature of the investigation, it lasted for some time. All of the results are now published, including two papers (44, 45) concerning the volatile substances. These writers believe that the alkalinity of the dew of the cotton plant is to be attributed, at least in part, to the presence of ammonia and trimethylamine, because these substances were found to be emanations from living cotton plants and were also identified in the dew collected from the foliage. Among the 12 individual substances found by them in the concentrated distillate, which included all the odorous and volatile constituents in the cotton plant, were 3 of particular interest for chemotropic tests. The so-called "essential" oil was about 0.003 per cent of the material employed. It had a strong, rather agreeable, and persistent odor. The ammonia and trimethylamine were present in appreciable quantities, but the ammonia largely predominated. So small an amount of trimethylamine as 0.0000005 gm. can be detected by its odor. In regard to the essential oil, ammonia, and trimethylamine, Power and Chesnut believe that only the trimethylamine serves as an attractant to allure boll weevils to cotton plants.

During the latter half of June, 1924, the writer used for the first time a sample of the concentrated and strongly odoriferous trimethylamine (33 per cent in water), which was isolated from cotton plants by Power and Chesnut. Several simple tests were made similar to those in which the sweet substances mentioned above were employed, but in no instance was any perceptible attraction observed. The next step was to develop a special apparatus to test this chemical further. A crude device, now greatly improved, and called by the writer an insect "olfactometer," was made. A brief summary of the results obtained in 1925 by the use of this instrument with other insects is given on pages 1135-1136. During June of 1924 only a comparatively few old weevils, which had come to the cotton fields from their hibernating places, could be collected daily, and it was therefore necessary to use the same weevils repeatedly until they failed to respond. Being thus handicapped by lack of material and with only a crude apparatus, the writer could not obtain accurate and positive results, but the preliminary laboratory experiments showed that when the trimethylamine was sufficiently diluted with water the weevils seemed to be slightly attracted.

Some of the old weevils mentioned above were put singly in small wire-screen cages with glass tops, the inside measurements of the cages being 5 inches long, 2 inches wide, and $\frac{1}{2}$ -inch deep. All the chemicals tested in these olfactory experiments were held in vials of equal dimensions and capacity. When a weevil was ready for testing, the stopper of one of the vials was removed and the vial was placed just beneath the weevil on the bottom of the cage. In each instance the source of the odor was about three-fourths inch from the weevil. In nearly all cases only the reaction times of the first responses observed have been recorded, the time being registered with a stop watch the seconds of which were divided into fifths.

Trimethylamine (1 to 100): 3 turned to one side quickly; 2 turned around over vial and flew away; 2 turned around over vial and walked away; 1 moved quickly and then flew; 1 moved slightly, then tried to get through wire screen over vial; 1 moved quickly, then tried to put snout through wire screen, and rubbed hind legs together; reaction time 2 to 10 seconds, average 3.9 seconds.

Trimethylamine (1 to 1,000): 4 moved away slowly; 2 turned around and rubbed legs together; 1 raised wings; 1 moved an inch toward vial and turned around over it; 1 moving weevil stopped 5 seconds after vial had been held under it, then tried to put its snout through wire screen over vial; 1 turned around over vial and tried to put snout through wire screen; reaction time 3 to 7 seconds, average 4.9 seconds.

Benzyl acetate: 5 moved away quickly; 2 turned around quickly; 2 jumped quickly; 1 moved away and worked legs. Reaction time 1 to 4.4 seconds, average 2.6 seconds.

n-Butyl acetate: 7 moved away quickly; 2 moved away slowly; 1 jumped quickly. Reaction time 0.6 to 5 seconds, average 1.9 seconds.

Methyl acetate: 4 moved away slowly; 4 moved away quickly; 1 worked legs; 1 moving weevil stopped, then walked away. Reaction time 2 to 6.4 seconds, average 3.5 seconds.

Allyl alcohol: 5 weevils tested; reaction time 4 to 10 seconds, average 6.2 seconds.

Capryl alcohol: 5 weevils tested; reaction time 2 to 7 seconds, average 4.3 seconds.

One weevil was tested with each of the following: Anisole, n-butyl alcohol, caproic acid, caprylic acid, carvacrol, citral, p-cymene, iso-amyl alcohol, iso-amyl benzoate, iso-amyl formate, iso-butyl acetate, iso-butyl alcohol, iso-propyl acetate, iso-propyl alcohol, methyl ethyl ketone, n-propyl acetate, n-propyl alcohol, and tertiary amyl alcohol, but no reaction was shown which indicated that the weevil was really attracted by the odor emitted.

CONCLUSION

Not one in the list of 24 chemicals tested, except the trimethylamine, seemed to have any attractive properties. The trimethylamine appeared to attract weevils slightly at times, but there was never any pronounced attraction. During these tests the vial was placed beneath a few moving weevils to determine what effect the odor would have on their movements. In most cases the insect stopped, very often rubbed its legs together, then feigned death, but several seconds after the vial had been taken away, it "awoke" and moved on.

Lutz (25, p. 275) tested a large number of chemicals found either in flowers or employed in the manufacture of perfumes. He says:

These were used with lard as a base in various concentrations and combinations. They were exposed in small dishes placed where flower-visiting insects were abundant, but no such visitors came to the dishes, although other insects (chiefly various flies) did so either by accident or otherwise. Possibly the right odors or combinations of odors were not used; possibly flower-visitors were not to be fooled by odors coming from such unflowerlike contraptions as glass dishes; and possibly insects do not find flowers by the sense of smell. Certainly, although the experiments consumed considerable time, they proved nothing.

SENSE ORGANS OF ADULT BOLL WEEVILS

Before entering into a discussion of the morphology of the sense organs of the boll weevil, it seems expedient to describe the material, methods, and histological technique employed in the study.

MATERIAL AND METHODS OF PREPARING WHOLE MOUNTS

The writer is often asked about his methods of preparing whole mounts of insects and about his histological technique, and since he has not published any details on these subjects in the past 10 years, he considers it worth while to give his most recent and best methods on these subjects, particularly since some of them have never been published.

Some of the material on the boll weevil was collected by the writer at Tallulah, La., in July and August, 1922; some in June, 1924; and other material was collected and preserved by various assistants of B. R. Coad, particularly by A. J. Chapman.

To obtain material for whole mounts of adult boll weevils, both dried and alcoholic specimens were used; but for whole mounts of larvae, only alcoholic specimens were employed. The specimens were treated with saturated caustic potash in the usual way by heating in a test tube over a Bunsen burner, or were allowed to stand in the unheated liquid for a day or longer, depending on their size and hardness. They were then removed and thoroughly washed with water, and the adult specimens were decolorized or bleached with chlorine gas in the following manner: A small quantity of potassium chlorate or chloride was put in a small wide-mouthed bottle (bleaching bottle). The specimens were then wrapped loosely in a small piece of cheesecloth which was suspended from a hook in the middle of the stopper. A pipetteful of hydrochloric acid was next dropped upon the potassium salt in the bottom of the bottle. At once chlorine gas was liberated, which in a few minutes bleached the dark-colored specimens. The specimens were again thoroughly washed in water and finally preserved in 50 per cent alcohol.

To study the external parts of the sense organs, 10 individuals of each sex and several larvae were dismembered by having all the appendages, head, thorax, and abdomen carefully severed. These parts were then put in vials in such a way that their identity was preserved. Some of these parts were temporarily mounted in 50 per cent alcohol, and others were mounted in Canada balsam or in a 15 per cent solution of potassium acetate. This solution, as well as the 50 per cent alcohol, gave a good refractive index, so that the minutest pores or slits could be easily discerned, but the balsam was less satisfactory in this respect. All parts were mounted between cover glasses in order that both sides of the specimen could be carefully studied under a high-power lens. In the case of the potassium-acetate mounts one cover glass was considerably smaller than the other in order that it might be securely sealed with asphaltum.

MATERIAL AND HISTOLOGICAL TECHNIQUE

To obtain material for the study of the internal anatomy of the sense organs, full-grown larvae and adult weevils (just ready to emerge) were taken from the flower buds, called "squares." To insure a good supply of material, a large number of squares bearing larvae and pupae were collected in the fields, and these were kept in the laboratory and examined daily. Some of the material was put at once in alcohol to be used for whole mounts, but most of it was preserved in the Bouin and the modified Carnoy fixing fluids. The latter, consisting of equal parts of absolute alcohol, chloroform, and glacial acetic acid, with corrosive sublimate to excess, was kept in a glass-stoppered bottle so that it might not lose its fixing ability by absorbing moisture from the air, although it does deteriorate within a few weeks in spite of all precautions taken. When material was dropped into vials containing this fluid, the stoppers were left off no longer than was absolutely necessary. To assure good fixation of the internal tissues, the snout or beak, antennae, head, legs, wings, and thorax were severed, and immediately dropped into the fixing

fluids, the Carnoy fluid being mostly used. The material preserved in the Bouin fluid was left in it overnight, and then washed several times in 95 per cent alcohol, but the material preserved in the Carnoy fluid was removed when it sank to the bottom of the vial, and then thoroughly washed in 95 per cent alcohol. All material was kept in 85 per cent alcohol until sectioned.

From this stage on, the procedure is considerably different from the double or paraffin-celloidin method described by the writer in 1915 (30). The successive steps in its use are as follows: Remove excess mercury in tissues fixed in Carnoy fluid by leaving pieces of material overnight in 85 per cent alcohol containing tincture of iodine (wine color); put small pieces of material containing sense organs in clear 85 per cent alcohol to wash out the iodine; put material in 95 per cent alcohol containing eosin; place pink tissue in absolute alcohol and ether (half and half) containing eosin, for only a few minutes; put material in tiny vials containing thin celloidin and leave overnight; next morning put vials in melted paraffin in small beaker on hot plate, and allow ether to boil gently for a few minutes; remove vials from paraffin, take out pieces of material, one at a time, and be sure that each has considerable celloidin surrounding it; drop each piece in chloroform and leave it until celloidin has become firm; remove these pieces from chloroform, dry on blotting paper, trim celloidin, and put them in paraffin in Petri dish on hot plate for a few minutes; when all bubbles have left them, transfer to tiny paper cups in another Petri dish containing the best and hardest paraffin (58° or 60° C., M. P.), and leave them there for a few minutes. (NOTE.—Never allow paraffin to become more than a degree or two higher than its melting point, for excess heat makes the tissue brittle.) With a very sharp knife cut the ribbons 5 microns in thickness, and if paraffin fails to ribbon properly, warm the knife; mount ribbons, using a thick film of fresh Mayer's albumen and flatten them by pressing on them with fingers, but use no water or heat, for heat blisters celloidin; let slides dry on top of paraffin oven 48 hours; after that put them in 50 per cent alcohol and then in Ehrlich's or Delafield's haematoxylin stain (well ripened) for three or four minutes; leave them in water one hour or longer, then run them up through the alcohols (50, 70, 85, and 95 per cent plus eosin), leaving them in each five minutes; then put them in clear 95 per cent alcohol to wash out the excess eosin; next in Gage's carbol-xylene (1 part carbohc acid to 3 parts of xylene) for only a few seconds, for this liquid slowly dissolves paraffin; lay them flat on match sticks upon the table, then drop xylene upon the slides gently with a pipette; after paraffin has dissolved, gently incline slides to allow xylene to run off them, then add Canada balsam and cover glasses.

Amateurs who try to follow these directions will doubtless have trouble, chiefly because experience and an understanding of the principles involved mean much more to a successful microscopic technician than the mere ability to follow directions closely. For this reason the writer hesitates to give such a complicated method, knowing that someone will pronounce it of no value.

OLFACTORY ORGANS

Since the sense organs of the adult or imago weevils are the more numerous and diversified, they will be described first, and a discussion of the sense organs of the larvae will follow.

Owing to the belief that the sense of smell in boll weevils is the primary one which aids them in locating cotton plants, the writer has paid more attention to the olfactory organs than to the other sense organs.

DISPOSITION OF OLFACTORY PORES

Hicks in 1857 (13) first described the sense organs herein referred to as "olfactory pores," and suggested that they might serve as olfactory organs. Nagel (42) saw some of them on the mouth parts of *Dytiscus marginalis*, and called them "Gruben ohne Kegel." Hochreuther (15) found them on the same species, and called them "Kuppelförmige Organe." He saw a few on the epicranium; 11 on the scape and pedicel?; a few on the labrum; very few on the mandibles; 6 to 8 on the maxilla; 14 to 16 on the labium; and 10 to 20 on the coxa, trochanter, femur, and tibia. Lehr, in 1914 (22), described them on the wings and elytra of the same beetle. Before this date they had been seen in other insects, and were called "organes sensitifs à ombelle" by Janet (20), and "sensilli campaniformi" by Berlese (1). Years ago the writer (28, 29, 30) studied them thoroughly in Hymenoptera and Coleoptera, called them olfactory pores, and determined to his own satisfaction that they serve as olfactory organs.

Several years ago J. A. Nelson, of this bureau, began a thorough study of the morphology of the boll weevil, but resigned before he had accomplished much. However, he prepared a 3-page manuscript on the sense organs, which still remains unpublished. Most of his time he spent in studying the disposition of the olfactory pores, but he also noted the tactile hairs and hypodermal gland pores. He found the olfactory pores located in about the same positions as recorded by the present writer in the following pages, and he saw a few very minute pores, presumably olfactory ones, on the tibial spines. In this insect the present writer did not note these, but has described them in the tibial spines of many other beetles. Nelson says, in part:

These pores are excessively minute, measuring 5-7 micra in diameter. Largely for this reason a study of their histological structure was found to be impossible.

* * * The most satisfactory sections were those of the pores at the bases of the antennae (of pupae). As far as could be determined, these correspond in structure to the so-called olfactory pores described by McIndoo, but the nature of the material makes it decidedly unfavorable for a study of the minute structures of these organs. It should be noted finally that in general the distribution and appearance of all of the sensory pores mentioned accord closely with the account given by McIndoo (1915). * * * Since McIndoo has brought sound experimental evidence to show that the pores described by him are actually olfactory in function in a wide range of insects, including several representatives of the Coleoptera, it would seem reasonable to extend this view to cover *Anthonomus*.

The elytra, wings, maxillae, and labium have dorsal and ventral surfaces; the submentum, only a ventral side; and for descriptive purposes, the antennae, legs, and mandibles may be divided into outer and inner surfaces. Since female No. 3 was studied most critically and all the drawings were made from this specimen, the disposition of its olfactory pores will be described in detail, and then will follow a discussion of the individual and sexual variations found in these organs in five females and five males.

Group 1, consisting of 53 pores (fig. 1, A, B), lies on the dorsal surface of the clytron, being found on the radial plate (RP) between the muscle disk (MD) and the subcostal head (ScH), with its distal

or broader end against the basal margin (BM) of the elytron. Groups 2 to 6 (C) lie on the wing, Nos. 2 to 5 being on the dorsal surface and No. 6 on the ventral surface; No. 2, of 25 pores, No. 3, of 30 pores, and No. 4, of 104 pores, lie on the radius (R); No. 5, of 4 pores, on the first cubitus (1Cu); and No. 6, of 26 pores, on the media (M).

Groups 7 and 8 and isolated pores *a*, *b*, and *c* are found on the legs (fig. 2); single pore *a* lies on either surface at the proximal end of the femur; *b* and *c* at the proximal end of the tibia, *b*, consisting of two widely separated pores, lies on the outer surface, and *c*, of a single pore, on the inner surface. Nos. 7 and 8 lie, respectively, on the outer and inner surfaces of the trochanter; No. 7 on the front legs consists of 5 pores, but of 6 pores on the middle and hind legs; No. 8 on the front and middle legs consists of 2 pores, but of 4 pores on the hind legs.

Groups 9 to 11 and isolated pores *d* to *n* are found on the mouth parts (fig. 3); *d*, *e*, and *f* (each of 1 pore), *g* (2 pores), No. 9 (4 pores),

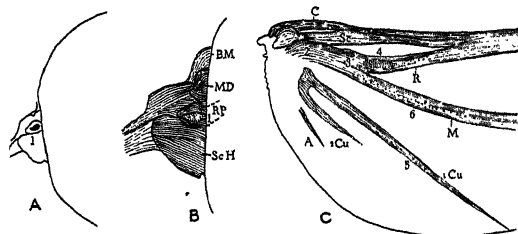


FIG. 1.—Portions of right elytron and right wing of female boll weevil No. 3, showing location of Groups 1 to 6 of olfactory pores as indicated by the numbers 1 to 6

A and B, peduncle of elytron, showing Group 1 on dorsal side of radial plate (RP); and C, showing Groups 2 to 5 on dorsal surface, and Group 6 on ventral surface of wing. A, $\times 19$; B, $\times 38$; and C, $\times 28$

Abbreviations: A, anal vein; BM, basal margin; C, costal vein; 1Cu, first cubital vein; 2Cu, second cubital; M, media; MD, muscle disk; R, radius; Sc, subcostal vein; and ScH, subcostal head

and No. 10 (5 slits) lie on the ventral surface of the maxilla, *d* being found on the stipes, *e* on the palpi, No. 9 on the first segment of the maxillary palpus, *f* on the second segment, and *g* and No. 10 on the third or distal segment of the same palpus (fig. 3, A and B). On the dorsal (partly lateral) surface of the maxilla are found the isolated pores *h*, *i*, and *j*; *h* (1 pore) lies on the palpi, *i* (1 pore) on the first segment of the maxillary palpus, and *j* (2 pores) on the third segment of the same palpus (C). On the submentum lies *k* of 5 pores, and on the ventral surface of the labial palpus lie *l* and *m*, each of 1 pore, *l* being found on the first segment and *m* on the third segment (D). The dorsal surface of the labium is devoid of pores (E). No. 11 of 4 pores and *n* of 2 pores lie on the outer surface of the mandible; the inner surface of the mandibles of this specimen bore neither pores nor hairs (F and G).

On the head capsule lie 10 pairs of pores (fig. 4, A and B); 2 pairs (*o*) are found just behind the compound eye on the epicranium; 3 other pairs (*p*) on the epicranium; 4 pairs (*q*) on the occiput; and 1 pair (*r*) on the genae (B).

At the base of one antenna lie 4 pores (C); 2 (*s*) being found on the outer surface and 2 (*t*) on the inner surface of the scape; but the other antenna of this specimen bears only 3 pores (A and B).

In regard to the individual variations only a few remarks need be made because most of the minor differences observed may have been due to the writer's inability to find the pores, particularly where the chitin was dark. As a rule, most of the groups of pores were fairly

constant in position, and varied only in the number of pores they contained. Some of the minor groups were often absent, or sometimes were united so that two were counted as one. No. 1 on the peduncle of the elytron was always present, and never had less than 45 pores. The 5 groups on the wings were sometimes reduced to 4, but the total number of pores was thereby seldom decreased; No. 5 ranged from 0 to 6 pores; No. 6 from 26 to 67; and in one instance No. 6 extended entirely to the fold in the wing. The disposition of

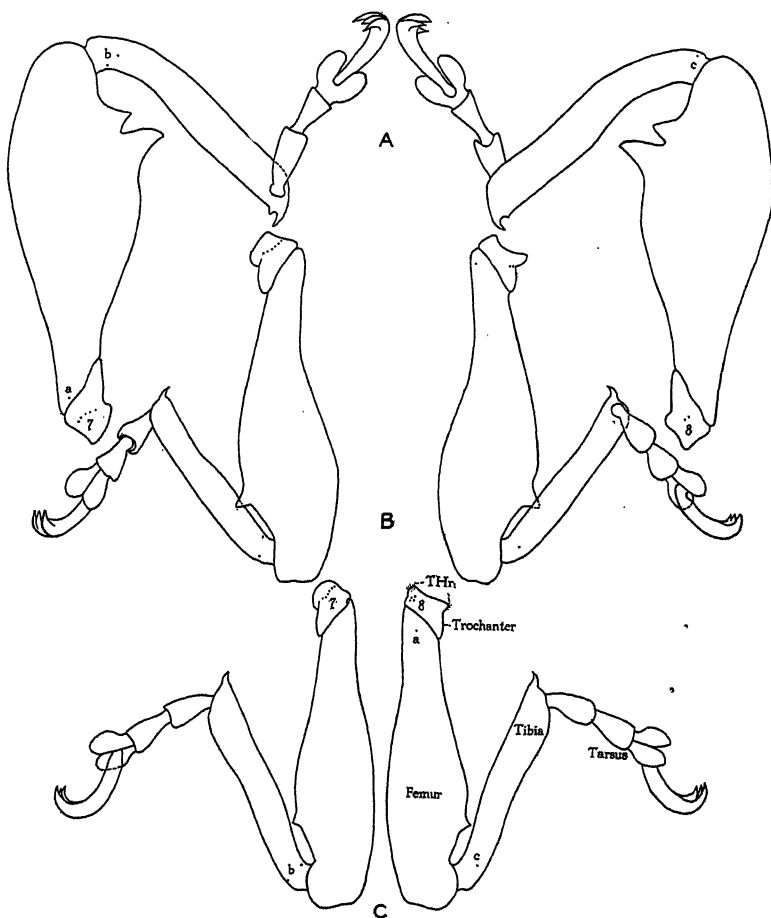


FIG. 2.—Portions of legs of female boll weevil No. 3, showing location of Groups 7 and 8, and isolated olfactory pores *a*, *b*, and *c*; tactile hairs (THr); A, front leg; B, middle leg; and C, hind leg. The drawings at the right represent the inner surface and those at the left, the outer surface. All $\times 24$

the pores on the legs, mouth parts, and antennae was similar to that already described, and the only striking difference found was in the total number of pores. Group 10 (fig. 3, B) on the third or distal segment of the maxillary palpus was always present and in each case seemed to consist of 5 slits. Only one male and one female were examined for pores on the head capsule (fig. 4, A and B), and no variations were noted; therefore probably all the head capsules bear these organs.

Relative to these pores, no sexual variations were found other than what might be regarded as individual differences, except that the females always had a greater number of pores than the males. Table 3 shows the individual and sexual variations in the total number of pores found. The pores varied in part as follows: Legs: Females, 22 to 28; males, 19 to 25. Elytra: Females, 90 to 106; males, 90

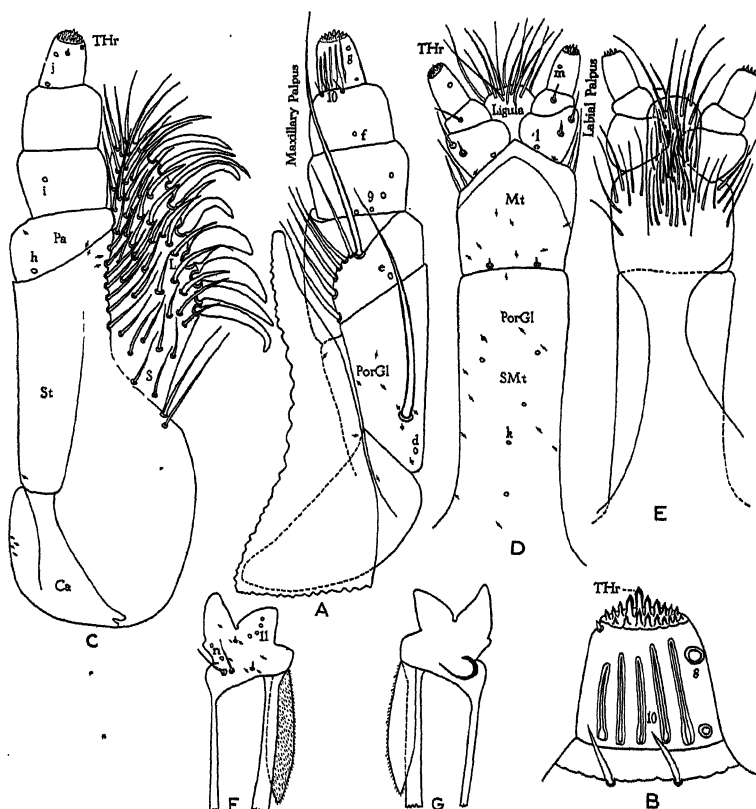


FIG. 3.—Portions of mouth parts of female boll weevil No. 3, showing location of Groups 9 to 11 and isolated olfactory pores *d* to *n*; tactile hairs (THr); and gland pores (PorGl). A, B, and C, maxilla; A, ventral surface; C, dorsal-lateral surface; and B, dorsal surface of distal or third segment of maxillary palpus. D and E, ventral and dorsal surfaces, respectively, of labium. F and G, outer and inner surfaces, respectively, of mandible. A, C, D, and E, $\times 200$; B, $\times 522$; and F and G, $\times 56$.

Circles and slits represent the olfactory pores, while black dots represent the gland pores, the lines through the latter showing the direction in which their efferent tubes extend. Ca, cardo; L, lacinia; Mt, mentum; Pa, palpifer; S, subgalea; SMt, submentum; and St, stipes.

to 96. Wings: Females, 376 to 413; males, 322 to 376. Total number: Females, 604 to 641; males, 527 to 576. Average: Females, 624; males, 549. Thus the females have 13.7 per cent more pores than have the males. It will also be noted that about two-thirds of the pores are borne by the wings, and nearly five-sixths by the wings and elytra combined.

EXTERNAL STRUCTURES OF OLFACTORY PORES

When examined under a low-power lens, the large olfactory pores may be easily mistaken for hair sockets from which the hairs have been removed; and the smaller ones may be mistaken for the pores of hypodermal glands, or vice versa. When they were more care-

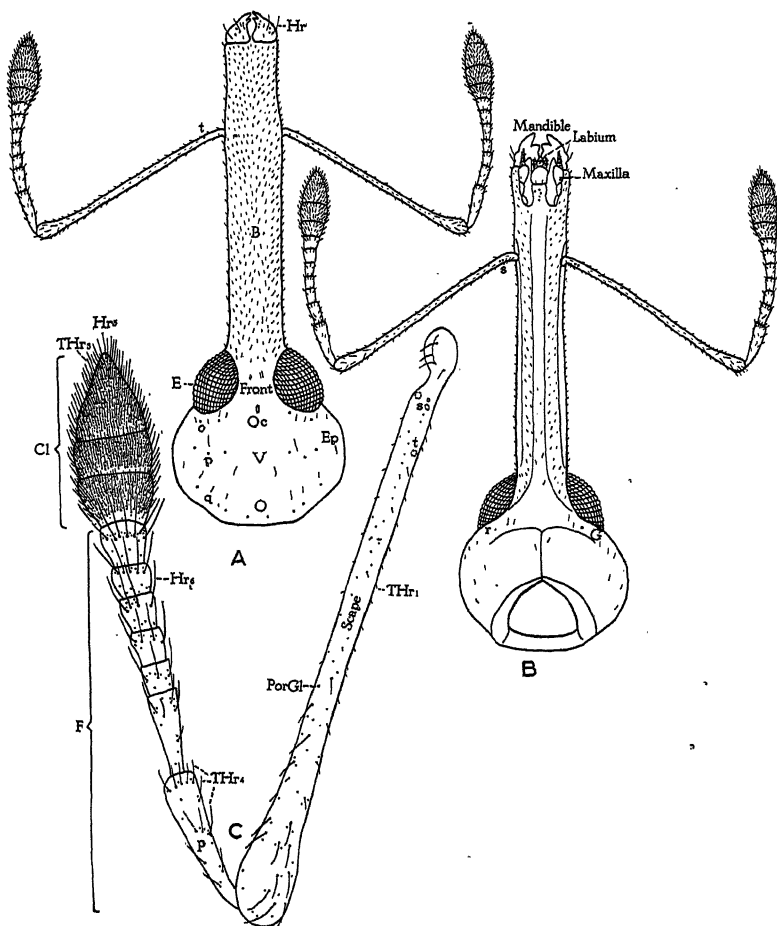


FIG. 4.—Head and its appendages of female boll weevil No. 4, showing location of olfactory pores (o to t), tactile or innervated hairs (THr, THr3, THr4), noninnervated hairs (Hr, Hrs, and Hrs4), and gland pores (PorGl). The disposition of olfactory pores is tolerably accurate but that of the hairs and gland pores is only approximate. In A and B the olfactory pores are represented by dots, but in C by circles (the dotted ones lie on opposite side of scape). In A and B the gland pores are not shown, but they are very numerous on the beak or snout, moderately numerous on the antennae, rather scarce on the mouth parts, and few on the head capsule; in C they are approximately located by dots. A, dorsal view of head and inner surface of antenna, $\times 24$; B, ventral view of head and outer surface of antenna, $\times 24$; and C, outer surface of left antenna, $\times 60$.

Abbreviations: B, beak or snout; Cl, club; E, compound eye; Ep, epicranium; F, funiculus; G, gena; O, occiput; Oc, ocellus; P, pedicel; and V, vertex.

fully observed under a high-power lens, it was usually not difficult to distinguish them from the hair sockets and gland pores. Both types of pores and hair sockets usually appear as small, round, bright spots when a strong transmitted light is used, but after a more careful study all three structures were found to differ widely. The hair

sockets generally appear the least bright; their walls are usually heavier and darker; and their pore apertures are usually bounded by rough or torn chitin, thus showing the mutilation caused by removing the hairs. The gland pores (fig. 5, A and B) as a rule are much smaller than the olfactory pores (fig. 5, C to L); they are many times smaller than those on the legs; usually much smaller than those on the wings and antennae; but almost the same size as those on the elytra and mouth parts. It is often difficult to distinguish a hair socket from an olfactory pore, but a gland pore is always easily recognized by its aperture (A, PorApGl) which, when one focuses downward on it, is observed to run V shaped from the pore wall (PorWGl) to the center or entirely across the pore.

TABLE 3.—Disposition of olfactory pores on adult cotton boll weevils

Appendages examined	Females					Males				
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 1	No. 2	No. 3	No. 4	No. 5
Front legs.....	22	25	22	22	22	23	22	20	22	22
Middle legs.....	24	26	24	24	24	22	19	24	24	24
Hind legs.....	27	25	28	28	28	22	25	22	22	22
Elytra.....	106	92	106	90	94	90	96	90	94	91
Wings.....	376	413	378	411	400	322	345	376	328	340
Mandibles.....	12	8	12	8	8	6	10	4	7	8
Antennae.....	7	7	7	6	7	6	6	6	6	6
Maxillae.....	23	36	36	29	36	27	23	25	26	27
Labium.....	5	4	4	4	4	4	4	4	4	4
Submentum.....	2	5	5	4	5	5	5	5	5	4
Total.....	604	641	622	626	628	527	555	576	538	548
	Average for females, 624 *					Average for males, 549 *				

* These numbers should be increased to 644 and 569 to include the 20 pores found on the head capsule each of a female and male.

The external structure of an olfactory pore consists of the aperture, wall, and border. The aperture (fig. 5, G, PorAp) may be round, oblong, or slit shaped (H). The wall (C, PorW) may be round, oblong (E), eye shaped (G), or slit shaped (fig. 3, B, and fig. 5, H). The border (fig. 5, G, PorB) may be round or oblong, but it was not discernible in most cases; on the wings it was usually present and surrounded each pore wall, although on the elytra it surrounded the entire group (fig. 5, E).

Most of the olfactory pores are round or oblong (fig. 5, C to E), but a few are eye shaped (fig. 5, F and G), and a few others are slit shaped (fig. 3, B, and fig. 5, H). The round or oblong ones are found on the femora, tibiae, elytra, wings, mouth parts, head, and antennae; the eye-shaped ones, on the wings; and the slit-shaped ones, on the trochanters and third segments of the maxillary palpi. In external structure the slit-shaped ones resemble the slits in the lyriform organs described in spiders by the writer (27) and others. Not all of those on the trochanters are as slender as the one shown (fig. 5, H), but they vary from this shape to the extremely elongated type.

INTERNAL STRUCTURE OF OLFACTORY PORES

In sections the olfactory pores are never mistaken for the gland pores (fig. 5, M to O) because they are usually much larger, and, besides, the reservoirs (Re) of the gland pores stand out quite con-

spicuously in the chitin. From the reservoir the efferent tube (Ef) leading to the exterior is also plainly seen, but the large gland cell (GIC) is not always easily distinguished from the hypodermal cells (HypC), although whenever it is discernible a portion of it runs into the pore canal (PorC) leading to the reservoir, and a clear area or ampulla (Am) in the gland cell lies just beneath the pore canal. These glands are almost like the young stages of those described in the squash beetle (*Epilachna borealis* Fab.) by the writer (31).

The olfactory pores (fig. 5, P to D') are more or less flask-shaped structures, and all really belong to one type, for as a rule their apertures open to the exterior on the same level as the surrounding chitin, and not into pits or at the tops of small domes as described by the writer (30) for some other beetles. However, the one represented in Figure 5, P, approaches the pit-shaped type, and the one in Figure

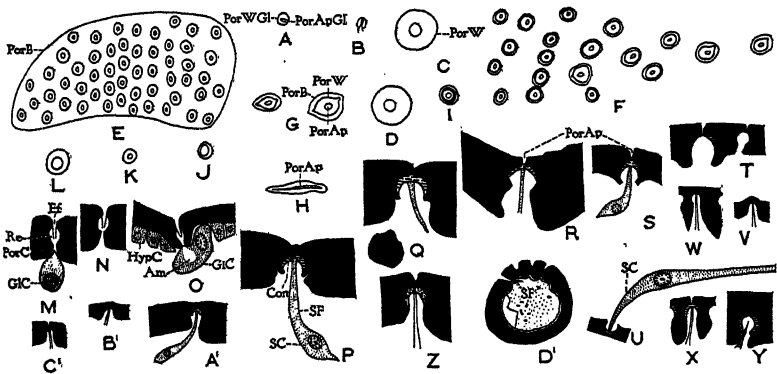


FIG. 5.—Cotton boll weevil: External structure of gland pores (A and B) and olfactory pores (C to L). A, from submentum; B, from antenna; C, from tibia of middle leg; D, from femur of middle leg; E, Group 1 on peduncle of left elytron; F, portion of Group 4 on wing; G, 2 pores from distal end of Group 4; H, one of slit-shaped pores on trochanter of hind leg; I, from first segment of maxillary palpus; J, from distal segment of labial palpus; K, from submentum; and L, from antenna. Internal structure of gland pores (M to O) and olfactory pores (P to D'). M and N, from antennae; M being from a section and N from a caustic-potash preparation, showing that the efferent tube (Ef) is chitinous. O, from ventral side of abdomen; P, from tibia; Q, from femur; R, from trochanter; S and T, from antennae, S being from a section and T from a caustic-potash preparation; U, from Group 4 on wing; V, from Group 6 on wing; W, from Group 2 on wing; X, from Group 1 on peduncle of elytron; Y, from head capsule (cut obliquely); Z, from mandible; A', from labial palpus; B', from stipes of maxilla; C', from second segment of maxillary palpus; and D', cross-section of third or distal segment of maxillary palpus.

Abbreviations: PorAp, aperture of olfactory pore; PorApG1, aperture of gland pore; PorB, border of olfactory pore; PorW, wall of olfactory pore; and PorWGL, wall of gland pore. Am, ampulla; Con, chitinous cone; GIC, gland cell; HypC, hypodermal cell; PorC, pore canal; Re, reservoir; SC, sense cell; SF, sense fiber. All $\times 438$.

5, V, the dome-shaped type. The chitinous cones (Con) were usually visible, but the sense cells (SC) were not always found. Owing to the difficulty of obtaining good sections and to the minuteness of these organs, it was impossible to find one in which all the parts were present. Figure 5, P, shows the sense fiber (SF) piercing the cone, while Figure 5, R and S, and others show the pore aperture (PorAp).

The third or distal segments of the maxillary palpi were repeatedly sectioned in order to arrive at an understanding of the internal structure of their five slit-shaped organs, but after all of these attempts it was not definitely learned how the sense fibers unite with the apertures. The difficulties encountered will be appreciated when it is understood that the diameter of this segment was the same

as that of a hair from the writer's wrist, and it was impossible to secure good fixation of its tissues. The best cross section made is represented by Figure 5, D', which shows that the knife passed through four of the five slits and that the aperture of one of these connects with the interior of the segment. It will be noted that one of the dark-staining bodies, the sense fibers (SF), unites with this aperture. Most of the sense cells present in the maxillary palpus lie in the first segment (fig. 6, A), while the remainder were seen in the second, but none was ever noticed in the third or distal segment. In the labial palpus (fig. 6, B) the sense cells are similarly located.

Figure 6, C, shows the large nerve (N) passing through the wing to group 4 on the dorsal surface. Attention is called to the sense cells (SC), trachea (Tr), thick and rigid chitin on the dorsal surface, but thin and flexible chitin on the ventral side. Figure 6, D, shows group 2 and other internal structures in a cross section of the wing.

ANTENNAL ORGANS

It will be noted that the above heading falls under "Olfactory organs." The antennal structures are described in connection with the olfactory organs because it is commonly believed that the antennae

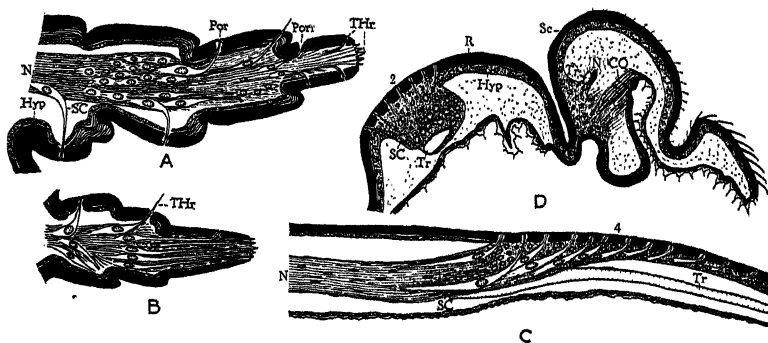


FIG. 6.—Cotton boll weevil: Semidiagrams, showing internal anatomy of maxillary palpus (A), labial palpus (B), and wings (C and D). A to C, longitudinal section; and D, cross section. C passes through Group 4, and D through Group 2

Abbreviations: CO, chordotonal organ ?; Hyp, hypodermis; N, nerve; Por, olfactory pore; Ppr, slit-shaped olfactory pore; R, radius; SC, sense cell; Sc, subcosta; THr, tactile hair; and Tr, trachea. A, C, and D, $\times 296$; B, $\times 494$

bear the organs of smell. The present writer, however, shows that the antennae do not bear organs suitable for this purpose, except the few olfactory pores at the base of each antenna. The antenna of the boll weevil is made up of three parts. The scape or scapus (fig. 4, C) consists of the first segment; the funicle or funiculus (F), of the second to eighth segments, but the second segment is also called the pedicel or pedicellus (P); and the club (Cl) consists of the ninth to twelfth segments.

The external anatomy of all the structures on the antennae of five males and five females was critically studied. Only three types of organs were found: (1) The 3 or 4 olfactory pores (Table 3) on the base of each scape (fig. 4, C), already described; (2) the gland pores, represented by dots widely distributed over the antenna; and (3) numerous hairs of various sizes. For descriptive purposes, the hairs are numbered from 1 to 6; but for comparative purposes, Nos.

1 to 4 are called sense hairs and sense bristles (Table 4). They are only approximately represented in Figure 4, C, and Figure 8, and in Table 4. The smallest ones, Nos. 1 and 2 (THr₁ and THr₂, fig. 7, A and B), were found almost entirely on the scape and club; the next in size, Nos. 3 and 4 (THr₃ THr₄, fig. 7, B and C), on all the segments, but mostly on the club; and the largest ones, Nos. 5 and 6 (Hr₅ and Hr₆, fig. 7, B and D), on the funicle and club.

The internal anatomy (fig. 8; fig. 9, A-G) of these hairs shows that all are innervated, except the largest ones, Nos. 5 and 6 (fig. 9, E and F). Numbers 2, 3, and 4 are most numerous, and those in the club seem to have a thread or extra fiber (fig. 9, C and D) running lengthwise in their sense cells from the base of the hair to the nucleus, or even beyond the nucleus. Figure 8 shows that the antenna is well supplied with nerves (N) and tracheae (Tr), but none of the innervated structures seems adapted to receive odor stimuli.

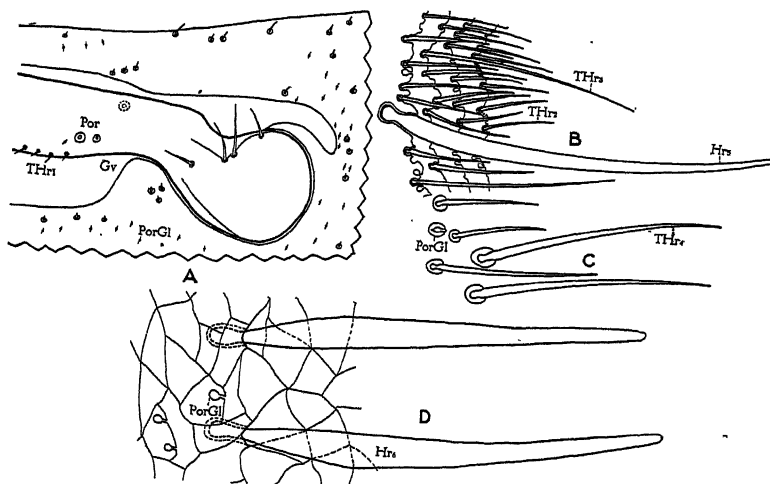


Fig. 7 (cf. fig. 4, C and fig. 8).—Cotton boll weevil: External structure of hairs on antenna and on beak at base of antenna. A, base of left scape lying in the antennal groove (Gv), showing tactile hairs, gland pores, and olfactory pores (the dotted one on opposite side); and B to D, besides showing hairs, show the antennal markings and gland pores

Abbreviations: Hr₅ and Hr₆, noninnervated hairs; Por, olfactory pore; PorGl, gland pore; and THr₁ to THr₄, tactile hairs. A, $\times 196$; B to D, $\times 510$

No experiments were conducted to determine whether the antennae of boll weevils carry the olfactory organs, but the writer in the past 10 years has tested many other insects having these appendages either cut off or otherwise mutilated. Since he has been criticized for using strong-smelling substances, chiefly essential oils, in order to induce the mutilated insects to respond to odors, he decided to conduct some experiments in which only the natural food odors would be used. Accordingly, two sets of potato beetles, each set of 50 individuals as usual, were tested in the insect "olfactometer" (38) by subjecting them to the odors from water extract, distillate, and emanations from living potato plants. The antennae were then cut off, and a few days later, when the insects had largely recovered from the operation, they were again tested daily with the odors and emanations. In all, 10 experiments, including 40 individual tests, were conducted. In all of the experiments except one the beetles

responded attractively to the plant odors, although slightly less so than before they were mutilated. Judging from these results alone and not considering the fact that the beetles had not totally recovered from the operation, we should say that the antennae bear only a few

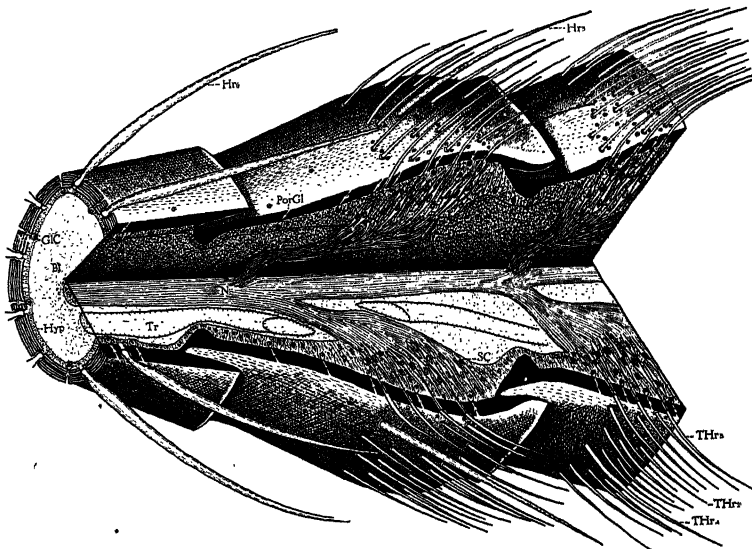


FIG. 8 (cf. fig. 4, C).—Cotton boll weevil: A schematic drawing of the ninth antennal segment with portions of the eighth and tenth, showing the antennal structures in perspective and in section. Note the following: Gland pores (PorGl), noninnervated hairs (Hrs and Hrs), innervated or sense hairs (THrs to THrs), none of which is suited for receiving olfactory stimuli. Bl, blood; GIC, gland cells; Hyp, hypodermis; N, nerve; SC, sense cell; and Tr, trachea.

of the olfactory organs, which is contrary to the general opinion held about the location of these organs. Nevertheless, Minnich (41), who conducted many experiments with cabbage butterflies, partly

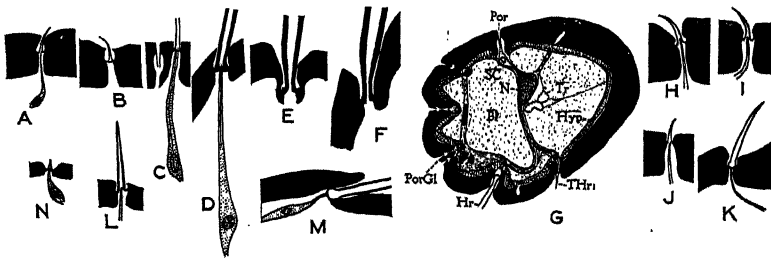


FIG. 9.—Internal structure of hairs of cotton boll weevil: A to D, innervated hairs, and E to F, noninnervated hairs on antennae. A is from a section, and B from a caustic-potash preparation, representing No. 1; C represents Nos. 2 and 3, and D, No. 4. Note the extra fiber in these two last sense cells. These fibers were only occasionally seen, but were not expected, considering the stain used. E represents No. 5, and F, No. 6. G, a cross section (semidiagrammatic) of base of scape passing through an olfactory pore (Por), a sense hair (THrs), a noninnervated hair (Hrs), gland pores (PorGl), and other structures; Bl, blood; Hyp, hypodermis; N, nerve; SC, sense cell; and Tr, trachea. H to N, tactile hairs from other parts of the boll weevil: H, from beak; I, from mandible; J, from head capsule; K, from thorax; L, from wing; M, from abdomen; and N, from ovipositor. A to F and H to N, $\times 490$; G, $\times 314$.

supports the view of the present writer. He found that the amputation of both antennae of these insects reduced the olfactory response 58 per cent, and, accordingly, claims that the antennae do not constitute the sole olfactory area.

TABLE 4.—Disposition of sense organs on head of adult cotton boll weevil

Head and its appendages ¹	Sense hairs, Nos. 1 and 2 (Sinneshaare)	Sense bristles, Nos. 3 and 4 (Sinnesborsten)	Sense pegs (Sinneszapfen)	Pit pegs (Grubenkegel)	Olfactory pores (Kuppelformige Organe)	Eyes	Johnston organ?	Chordotonal organ
Head capsule.....	Numerous on beak, especially near mouth parts and base of antenna.	Few on epicranium, vertex, front, occiput, genae.	Few near base of antenna.	None.....	10 on epicranium, 8 on occiput, 2 on genae.	2 compound eyes, 1 rudimentary ocellus.	-----	-----
Antenna.....	About 51 on scape, 2 on funiculus, 280 on club.	About 12 on scape, 20 on funiculus, 200 on club.	None.....	do.....	3 or 4 on base of scape.	-----	In pedicel.	In pedicel.
Mandible.....	2 on outer surface.	None.....	do.....	do.....	4 to 12.	-----	-----	-----
Maxilla.....	2 on second segment of palpus.	Probably many on pal- pifer and lacinia.	19 at tip of palpus.	2 near tip of pal- pus.	1 on stripes, 2 on palpifer, 13 on palpus.	-----	-----	-----
Labium.....	2 on mentum, 3 on palpus.	Probably many on men- tum and ligula.	9 at tip of palpus.	None.....	5 on submentum, 2 on palpus.	-----	-----	-----

¹ The labrum is absent in the boll weevil.

TACTILE ORGANS

Besides the tactile hairs (Nos. 1 to 4) already described on the antennae (fig. 9, A to G), innervated hairs were also found elsewhere; particularly on the mouth parts, other appendages, and even widely distributed over the body. The only safe way to determine a sense hair is to find its nerve in sections, but this is a difficult and slow task, and one can hardly be expected to do this for every individual hair; so after a careful study of the internal anatomy of certain groups had been made, the others were judged merely from their external structure. The internal anatomy of only a part of those recorded in Tables 4 to 6 were studied in sections, but since other investigators have found tactile hairs similarly located in other beetles, the writer feels that most of those which he calls sense hairs are really innervated, although perhaps he has overlooked certain other ones.

DISPOSITION OF TACTILE HAIRS

Hochreuther (15) made a thorough study of the sense hairs on a water beetle (*Dytiscus marginalis* L.). On the basis of external structure, he separated them into five divisions, one of which will be discussed under another heading. Since the writer knows of no similar work pertaining to a weevil, he has used Hochreuther's classification as a guide in the present investigation.

TABLE 5.—Disposition of sense organ on thorax of adult cotton boll weevil

Divisions of thorax and its appendages	Sense hairs	Sense bristles	Sense pegs	Pit pegs	Olfactory pores	Chordotonal organ
Prothorax.....	None.....	Several on margins of pronotum and post-sternum.	None.....	None.....	None.....	
Mesothorax.....	Numerous on anterior margins of episternum and mesosternum; on prescutum and scutellum.	Same as for sense hairs.	...do....	...do....	...do....	
Metathorax.....	Several on anterior margin of metatergum.	Several on metatergum, metapleurum, and metasternum.	...do....	...do....	...do....	
First pair of legs..	Several on coxa; 2 groups on trochanter; several at distal end of femur and on its teeth; few on tarsus.	Several on coxa; several widely distributed on femur, tibia, and tarsus.	...do....	...do....	6 to 8 on trochanter; 1 on femur; 3 on tarsus.	
Second pair of legs.	Several on coxa; 2 groups on trochanter; remainder, same as above, but hairs less numerous.	Distribution same as on first pair of legs, but less numerous.	...do....	...do....	6 to 9 on trochanter; 1 on femur; 3 on tarsus.	
Third pair of legs.	Same as above....	Same as on second pair of legs.	...do....	...do....	7 to 10 on trochanter; 1 on femur; 3 on tarsus.	
Elytra.....	None.....	None.....	...do....	...do....	45 to 53 on each peduncle.	
Wings.....	Few.....	Few.....	...do....	...do....	322 to 413 on both wings.	In base?

SENSE HAIRS NOS. 1 AND 2 (SINNESHAARE, or SENSILLA TRICHODEA).—Hochreuther found these on the head capsule, antennae, all mouth parts, thorax, legs, abdomen, and sexual apparatus. The present writer found them on the head and all its appendages (Table 4), parts of thorax, legs, and wings (Table 5), and on certain parts of the abdomen (Table 6).

SENSE BRISTLES, NOS. 3 AND 4 (SINNESBORSTEN, or SENSILLA CHAETICA).—Hochreuther found these on the epicranium, antennae, labrum, maxillae, labium, epipharynx, cervical sclerites, thorax, legs, and abdomen. The present writer found them on the head capsule, antennae, and probably on the following: Maxillae, labium, thorax, legs, wings, and abdomen (Tables 4 to 6).

TABLE 6.—Disposition of sense organs on abdomen of adult cotton boll weevil

Divisions	Sense hairs	Sense bristles	Sense pegs	Pit pegs
Tergites.....	Few on each.....	Few on each.....	None.....	None.
Pygidium, or last tergite.....	Few.....	Few.....	Few.....	Do.
Female genitalia.....	None.....	Several on palpus of ovipositor.	Few on palpus of ovipositor.	Many on ovipositor.
Male genitalia.....	None.....	None.....	None.....	Very numerous on body (or penis).
Sternites.....	Very numerous on last sternite; less numerous on other sternites.	Same as for sense hairs.do.....	None.
Epipleurites.....	Few on each, near spiracle.	None.....do.....	Do.

SENSE PEGS (SINNESZAPPEN, or SENSILLA BASICONICA).—Hochreuther found these on the head and all its appendages, thorax, legs, abdomen, and sexual apparatus. The present writer found probably a few on the head, maxillae, labium, and female genitalia.

PIT PEGS (GRUBENKEGEL, or SENSILLA COELOCONICA).—Hochreuther found these on the antennae, all mouth parts, epipharynx, mesothorax, legs, and sexual apparatus. The present writer found two near the tip of the maxillary palpus, and many on the female and male genitalia.

STRUCTURE OF TACTILE HAIRS

Vom Rath (47, 48) found sense cells connected with all the small hairs on the maxillary palpi of *Coccinella septempunctata*, *Melolontha vulgaris*, and *Tenebrio molitor*, and also with all the small hairs on the labial palpi of the last species, in much the same manner as shown in Figure 6, A and B. Using Ehrlich's "Methylenblau method" and Golgi's "Chromsilberverfahren," Vom Rath traced the sense fibers of insects, myriapods, spiders, and crustaceans. In all the innervated hairs, whether short or long, and even in those at the tips of the maxillary and labial palpi, he saw a fine thread running lengthwise through each sense fiber to the tip of the hair, as shown in Figure 9, C and D, although the present writer was able to trace the sense fibers only to the base of the hair and never far into its cavity. Relative to Vom Rath's work, Hilton (14, p. 566), who has reviewed the literature on this particular subject, says:

Earlier workers with methylen blue who studied simply the surface views represent nerves coming from the tips of the hairs; but it seems probable that

such figures are in large part diagrammatic. Vom Rath found by the Golgi method cavities of sensory hairs filled with nerves; this result is regarded as an artifact by Duboseq, who shows clearly how appearances like nerves may be obtained in the cavity of hairs due to deposits of chromate of silver; and he shows quite clearly that when nothing but the nerve cell and fiber is impregnated the nerve fiber stops at the base of the hair, as was apparently the case in his methylen blue preparations.

Figure 9, A to N, illustrates internal structure of hairs as seen by the writer, using Ehrlich's haematoxylin stain with eosin.

THE SO-CALLED TASTE ORGANS

Several writers, particularly Nagel (42), have described certain tiny peglike hairs on the mouth parts of insects as taste organs, but no one has ever demonstrated that they perform such a function. Hochreuther (15) found many "Tast- und Geschmackszäpfchen" on the maxillary and labial palpi of *Dytiscus marginalis*. Comstock (5, p. 132) briefly summarized the description of such hairs, and said:

Many experiments have been made to determine the function of the various chemical sense-organs but the results are, as yet, far from conclusive.

The present writer wishes to emphasize the fact that so far as he is aware no recent observer has found gland cells connected with these sense hairs, as claimed by Berlese (1).

As shown for adult boll weevils in the preceding pages and for the larvae in the following pages, only two kinds of sense organs—olfactory pores and innervated hairs—were found on the mouth parts. Neither of these seems suitable to act as taste organs, and consequently the writer has come to the same conclusion concerning the boll weevil that he reached several years ago (32) in regard to the honeybee. In brief, he does not believe that insects have a true gustatory sense, but since in many cases they must first "taste" certain foods before they can discriminate between them, it seems that their responses may be similar to ours when we are "tasting" the flavors of particular foods. Long ago physiologists determined that flavors to us are only smells, brought about by placing food in the mouth and then exhaling through the nose.

Since it was impossible to demonstrate by experiment the function of the sense organs on the mouth parts, the writer argued that in view of the fact that bees do not have suitable gustatory organs they can not have a true gustatory sense. Snodgrass (54, p. 59-63) well summarizes the writer's results, and adds the following precautionary remarks:

Most entomologists, probably, will not be convinced by this form of reasoning that insects do not taste, since it places too much reliance on a personal interpretation of the functional possibilities of an observed anatomical structure. It shows, however, that the experimenter who claims a sense of taste for insects must demonstrate it with tests that will clearly avoid a confusion between taste and smell.

THE SO-CALLED AUDITORY ORGANS

Much has been written about the auditory sense of insects, but critics still contend that it has never been demonstrated beyond a doubt that any insect can really hear. Most students on insect behavior believe that insects can hear, yet very few have produced evidence supporting this belief. Rádl (46), after summarizing the results of several other investigators on this subject, says that certain

experiments have convinced him that insects have a crude auditory sense. He offers several reasons for this conviction, and concludes by saying that the auditory sense in insects is on a much lower plane of development than that of vertebrates. Its anatomical and physiological antecedents are to be found, not in the tactile organs and contact activities, but anatomically in sense organs which register muscle activities and physiologically in general sensation. That is, he believes that their sense of hearing is a highly refined muscular sense. Turner and Schwarz (56) and Turner (55) have produced good experimental evidence to show that certain moths really hear, although the present writer is unaware of results equally convincing that beetles can hear; nevertheless, it is common for them to have stridulating devices and chordotonal organs.

The common belief that insects can hear is based on the three following facts: (1) Many have special sound-producing organs; (2) some have so-called auditory organs; and (3) many of the experimental results obtained indicate that insects can hear, although certainly not as we do.

Since the writer could find no reference in literature to the "auditory" organs in a weevil, he has used the two investigations of Lehr (22, 23) as a guide for the work here reported. Lehr, who first studied the anatomy of the sense organs in the elytra and wings of *Dytiscus marginalis*, found a chordotonal organ at the base of the subcostal vein. It lies on the ventral side beneath a group of olfactory pores, is well developed, and has all the parts common to such an organ. In his second work (23), Lehr pointed out two other so-called auditory organs in the pedicel or second antennal segment. The Johnston's organ is situated near the distal end of the segment; while the chordotonal organ, lying by its side, arises nearer the proximal end. Both of these organs run lengthwise in the segment and connect with the articular membrane between the second and third segments, but Lehr failed to see all their parts distinctly. Judging from his drawings, the Johnston's organ is the better developed. At this place in the antenna the interior is nearly filled with sense cells.

Johnston in 1855 (21) first pointed out a supposed auditory organ in the second antennal segment of the *Culex* mosquito. This structure, later called after his name, was thoroughly investigated by Child (3), who saw it in all the insect orders examined, except the Orthoptera. He found it in Diptera, Hymenoptera, Coleoptera, Neuroptera, Pseudoneuroptera, and Homoptera. He also saw sense organs in the second antennal segments of Orthoptera, but decided that they were not Johnston's organs. These have since been described as olfactory pores by the present writer (36), who has also recently described the Johnston's organ in the honeybee (37). He did not, however, see a chordotonal organ in the same segment, as mentioned by Lehr and as here reported for the boll weevil.

Externally, there are no signs of an auditory organ in the pedicel or second antennal segment (fig. 4, C) of the adult boll weevil, but sections through the distal end of this segment always show large masses of sense cells. After these masses had been carefully studied under the highest magnification, they were divided into two groups, based on size and structure; but, owing to their minuteness and perhaps to poor fixation and staining, it was not possible to distinguish

all the details ordinarily described for the supposed auditory organs. Since these groups are similar in position and somewhat resemble those described by Lehr in *Dytiscus*, the writer has called one of them the Johnston's organ (fig. 10, A, J) and the other the chordotonal organ (CO), although perhaps these names should be reversed, or possibly both groups belong to the same organ.

Both groups of sense cells run nearly the full length of the segment. Near the distal end (fig. 10, A) they expand and occupy most of the interior, but near the proximal end (fig. 10, B) they are much smaller and take up less space. One side of each group is always in contact with the hypodermis and seems firmly anchored. The distal ends of

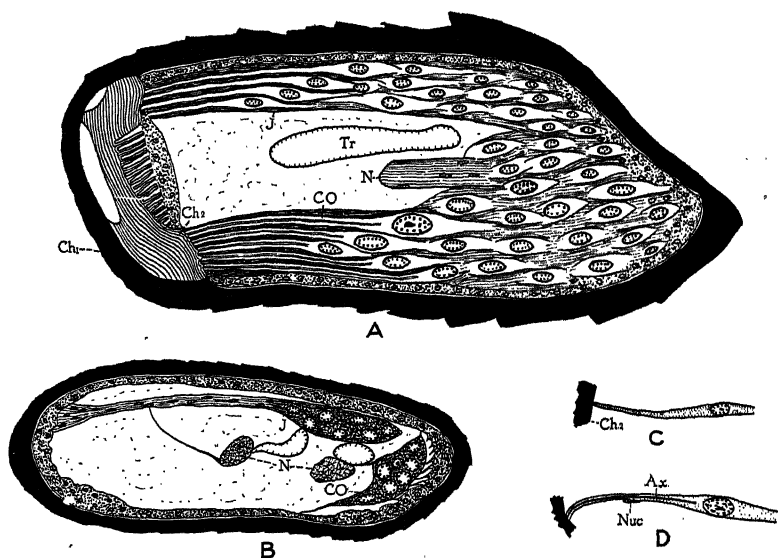


FIG. 10.—The so-called auditory organs of the cotton boll weevil. A and B, two-thirds diagrammatic from oblique longitudinal sections of pedicel (*P* of fig. 4, C), showing Johnston's organ (J) and the chordotonal organ (CO). A, from distal end of segment and B, from proximal end. C and D, two of smallest cells from these organs, C being from Johnston's organ and D, from the chordotonal organ. $\times 564$

Abbreviations: Ax, axial tube; Ch₁, primary cuticle; Ch₂, secondary cuticle; N, nerve; Nuc, nucleus; and Tr, trachea

the sense cells are attached to the secondary cuticula (fig. 10, A, Ch₂) of the articular membrane between the second and third segments. A nerve is always near the groups, and branches of it (N) can be traced directly to the sense cells.

The sense cells in the Johnston's organ are usually smaller than those in the chordotonal organ, and seem to be simple in structure (fig. 10, C), while each sense cell in the chordotonal organ apparently bears two additional structures—the axial tube (fig. 10, D, Ax) and secondary nucleus (Nuc). This would seem to indicate that the chordotonal organ consists of the various parts common to this type of sense organ.

As already stated, Lehr found a well-developed chordotonal organ in the base of the subcostal vein of *Dytiscus*. The present writer likewise found a chordlike structure (fig. 6, D, Co) in identically the same place in the boll weevil, but no details could be distinguished.

Since it appears nearly structureless in the few sections showing its presence, we may infer that it is either a rudimentary organ or had not had time to develop fully when the weevils emerged.

Grabner (10), after finding chordotonal organs in many of the insect orders, was convinced that the integument of insects, like the tympanum of vertebrates, is well adapted to be set in vibration by sounds, and that the terminal nerve connections of these organs are the sound receptors.

During the past 75 years, the stridulating or sound-producing organs of insects have been much discussed, but still we do not know whether the noises made by them are intentional or unintentional. Darwin (7) calls these organs sexual characters, but he has much difficulty in finding examples to prove that they are so. If all our present information were carefully analyzed, however, with this object in view, we might be able to produce many good examples to support his belief.

In regard to beetles Darwin (7, p. 310, 311, 312) says:

Nevertheless, the power of stridulating is certainly a sexual character in some few Coleoptera. * * *. In the case of the *Heliophanes* and *Oryctes* there can hardly be a doubt that the males stridulate in order to call or to excite the females; but with most beetles the stridulation apparently serves both sexes as a mutual call. Beetles stridulate under various emotions, in the same manner as birds use their voices for many purposes besides singing to their mates. The great *Chiasognathus* stridulates in anger or defiance; many species do the same from distress or fear, if held so that they can not escape; by striking the hollow stems of trees in the Canary Islands, Messrs. Wollaston and Crotch were able to discover the presence of beetles belonging to the genus *Acalles* by their stridulation. Lastly, the male *Ateuchus* stridulates to encourage the female in her work, and from distress when she is removed. Some naturalists believe that beetles make this noise to frighten away their enemies; but I can not think that a quadruped or bird, able to devour a large beetle, would be frightened by so slight a sound * * *. Finally, it is probable that the two sexes of many kinds of beetles were at first enabled to find each other by the slight shuffling noise produced by the rubbing together of the adjoining hard parts of their bodies; and that as those males or females which made the greatest noise succeeded best in finding partners, rugosities on various parts of their bodies were gradually developed by means of sexual selection into true stridulating organs.

So far as known to the writer, no one has reported stridulating organs in boll weevils, nor has anyone heard noises made by these insects, yet stridulating organs may be present and the adults may make noises which have never attracted the attention of any observer. Darwin cites cases of other curculionids having stridulating organs with which they are able to make audible noises, and Westring (58) as early as 1847 reports finding these organs in certain curculionids. In these weevils the rasps are located on the inferior surface of the elytra, near the apices, or along their outer margins, and the edges of the abdominal segments serve as the scrapers. This arrangement is the reverse of what usually occurs in other beetles. These weevils make a loud noise by rapidly rubbing the tips of the abdomen on the ends of the elytra. As already quoted by Darwin, Wollaston (59) called *Acalles argillosus* in the Canary Islands a musical curculionid, and another observer reports five other species of *Acalles*, one of which is very minute, as being musical in the Madeira Islands. All of these species have stridulating organs.

EYES AND LIGHT EXPERIMENTS

The two compound eyes (fig. 4, A, *E*) of the adult boll weevil are present; but, as reported by other writers, the ocelli are apparently absent. The faceted eye is small, though moderately convex. The number of facets in the eyes of one male and one female were counted; they ranged from 400 to 448, with an average of 433 per eye; and there was practically no sexual difference. According to Müller's mosaic theory of sight in insects, which most entomologists have accepted, the larger and more convex the eyes the larger will be the visual field, and the smaller and more abundant the facets the sharper and more distinct will be the image. Using the preceding information as a basis for speculation, we may infer that the visual field of the boll weevil is small and its image of objects is neither sharp nor distinct. From this statement we may also infer that the boll weevil probably can not, merely by sight, distinguish from a distance cotton plants from certain other plants, and furthermore that it may not be able by sight alone to distinguish the various parts of a cotton plant.

Many sections of the eyes were made, but after a preliminary study of them no unusual structures were noted that would modify the generally accepted view of insect vision. If one may judge from the large amount of pigment present, the boll weevil has day eyes and not night eyes. The subject of day and night eyes is well summarized by Comstock (5, p. 142).

A median ocellus (fig. 4, A, *Oc*) was finally found in the boll weevil after a diligent search under a high magnification. It is very small, extremely oblong, and lies nearly buried in a slit between the compound eyes; but viewed from the inner side of caustic-potash preparations it appears much larger, and is nearly round.

The only light experiments conducted by the writer were a few preliminary ones to verify the statement that the boll weevil is positively phototropic to daylight. No artificial lights were tested; although it might be possible to attract weevils to lights closely imitating daylight. Ever since the advent of this insect to cultivated cotton fields, and even up to the present time, there has been a belief that it might be attracted to artificial lights and then killed. Hunter and Hinds (17, p. 160) and Hunter and Pierce (18, p. 154) assure us that there is no scientific foundation for this belief. Thousands of insects, including many weevils similar in appearance to the boll weevil, have been attracted to lights in cotton fields, but as yet no scientist has found a single cotton boll weevil among them.

Hunter and Pierce (18, p. 43) report the results of some preliminary experiments conducted in 1907 to ascertain the color sense of boll weevils. Of the 14 shades and colors used, the 3 most attractive were light blue, dark green, and light pink, the light blue being most preferred.

Lutz (25, p. 265), discussing the color sense of insects, says:

Hess may be right in believing that insects are totally color blind. Probably Frisch is more nearly correct in saying that they can distinguish all of the colors except red and certain greens as colors, those two of our colors appearing to them as darker or lighter grays; in other words, that their color vision is similar to that of some partially color-blind humans.

Lutz determined that the flowers and occasionally other parts of certain plants emit ultra-violet rays, and that flower-visiting insects respond to ultra-violet light, but he did not use the cotton plant

and the boll weevil; although it is possible that the cotton plant emits these rays and perhaps the boll weevil responds to them. Lutz (25, p. 271, 278, 280), further states:

It has been shown that, in addition to the colors which man can see, some flowers are ultra-violet, while others are not at all ultra-violet, and still others have an ultra-violet pattern. It has also been shown that flower-visiting insects can see ultra-violet as well as or even better than they can see the rays perceived as light by man. The effect of this work is to add one more color—and apparently an important one—to the list of those which plants use in facilitating the visits of insects, if plants do use colors for such a purpose.

All of the colors of the spectrum from red to ultra-violet, both included, are to be found in light reflected by one flower or another. Of these waves of light reflected by flowers, those of relatively great length, red to green, are more common than those of shorter length, blue to ultra-violet. Flower-visiting insects do not see red to green as well as they do blue to ultra-violet.

It is also shown that a wide range of flower-visiting insects are photopositive to ultra-violet. It appears as though they see ultra-violet better than they do colors that seem bright to us. Accordingly, ultra-violet becomes quite as important in discussions of the relation between floral colors and insects as any other color.

OTHER SENSES

Among the general sensations of insects might be mentioned those of temperature, humidity, direction, hunger, fear, pain, and statical conditions. Very little is known about these senses in insects in general, and practically nothing about them in the boll weevil.

The sense of temperature in the boll weevil, judging from its reactions to different degrees of heat and cold, is probably well developed, but, as in other insects and even in ourselves, there are probably no special sense organs to receive thermal stimuli. The subhypodermal nerve plexus, if it is present in the adult weevil, could easily perform this function. Humidity, which is closely related to temperature, also has much to do with the behavior of this insect.

In this connection might also be mentioned some of the ingenious but absurd mechanical devices which have been invented from time to time for the purpose of eradicating the boll weevil. Among these the most common are "light" traps, one even being pulled through the fields on wheels; another is planned to attract the weevils by sounds; and still another would kill the weevils with a beam of high pitched inaudible sound waves of great intensity.

One of the newest ideas about the means of communication among insects is wireless telegraphy. This suggestion seems to have been first made by Fabre (8, p. 194-199) to explain how male moths find their mates from long distances, but his experiments convinced him that moths do not communicate by this means; next he predicted that science would provide us with a radiography of odors, after the pattern of the Röntgen rays, although Forel (9) says that Fabre's own results contradict this view. The next idea along this line was suggested by Riley (50, p. 38-41), who used the word telepathy to explain how insects communicate from long distances, but still he had no faith in this view.

Now, since we are able to communicate among ourselves so readily by radio, this means has been suggested as possible for insects. The writer can find no authentic data on this subject, but in some newspaper reports which recently appeared it is stated that one authority claims that man and most other animals constantly emit

N rays or electric rays which are used in communication. He says in part:

Look at the birds which fly at night, insects, blind fish at the bottom of the sea—all are irresistibly drawn toward their prey not so much by sight or odor, but by the action of the radiations which direct them and to which they submit automatically.

In regard to insects he imagines that these tiny "radio waves" are received by the antennae. Another report, this one given out by Science Service, of Washington, D. C., says in part:

Animals and plants while living give off rays similar to those emitted by the metal radium, according to reports made to the French Academy of Sciences by Albert Nodon.

Mast (39) experimented with fireflies and concluded that smell played no part in bringing the sexes together, but that the sexes communicate and are attracted to each other by means of their flashes of light. Singh and Maulik (52) determined that the so-called "phosphorescence" of the fireflies would affect the photographic plate through wood, dark brown leather, black paper, or flesh, and conclude:

The light emitted by the insect cannot therefore be taken as phosphorescent. It may be, perhaps, premature to conclude that some of the rays emitted by the insects are X-rays, but it may be safely asserted that these rays are, at least, similar to X-rays and ultra-violet light in so far as they render certain opaque media transparent and are intercepted by glass.

Harvey (11, p. 61, 62) says:

Although Muraoka (1896) and Singh and Maulik (1911) have described radiations coming from fireflies which would pass opaque objects and affect a photographic plate, * * * the existence of such radiation has been denied by Suchsland (1898), Schurig (1901), and Molisch (1904).

There is, then, no specific emission of X rays or similar penetrating radiation from luminous tissues which will affect the photographic plate through opaque screens.

A letter from E. N. Harvey to the writer, dated June 7, 1925, contains his latest ideas on this subject, including the possibility that boll weevils might give off rays which can be perceived by themselves but not by us. He states:

I do not think there is any evidence that animals give off X rays or any other kind of peculiar radiation. Short ultra-violet radiation is very destructive to living matter, so there is not much chance of wave length shorter than 3,000 Å being emitted, but I see no reason why ultra-violet between 4,000 Å and 3,000 Å should not be produced. We do not know definitely of a case, so that the matter is merely speculation.

RECOGNITION AMONG BOLL WEEVILS

Hunter and Hinds (17, p. 76) found that the male boll weevil was unable to recognize the female at a much greater distance than an inch, and that the attraction is apparently effected by smell. Field observations, as well as laboratory ones, seemed to show that the sexes are attracted only when they meet, as they are likely to do either on the stems or squares of the plant.

While watching female weevils both in the field and in confinement, the writer observed that they seemed to pay little attention to one another; but such is not usually the case where the opposite sex is concerned. The males in confinement usually do not lose an opportunity to mate, and should no female be handy, a male may often be

seen trying to copulate with another male. This behavior of either sex does not necessarily imply that the females do not know one another, or that the males can not distinguish the sexes, because we can cite many similar cases among other animals; for example, cows in heat or rut often try to copulate with one another.

If boll weevils do not communicate with one another by telepathy, radio, or by any other type of radiation, as mentioned above, then there are left perhaps only three other means—sight, touch, and smell. At short distances the weevils may be able to distinguish many other insects and perhaps inanimate objects by sight alone, and when in contact may partially recognize one another by sight and touch; but if they are similar to other insects and the higher animals, smell probably plays a more important rôle in recognition than do the other senses.

If boll weevils recognize one another by means of smell, then they must produce odors which can be smelled. Insects, as well as the higher animals, have glands connected with their sexual organs, and those in the boll weevil might serve for sexual recognition.

The unicellular glands found widely distributed over the entire body surface are, in point of abundance, as follows: On the elytra (fig. 16, E, *po*) and antennae (fig. 4, C; fig. 8, *PorGl*), very numerous; on the beak or snout (fig. 7, A), numerous; on the wings (fig. 16, D, *po*) and ventral side of the abdomen, fairly numerous; on the head capsule, mouth parts (fig. 3, A, C, D, and F), thorax, legs (fig. 16, C), and dorsal side of the abdomen, few.

The external appearance of these glands is shown in Figure 5, A and B, and the internal anatomy in Figure 5, M, N, O, and Figure 16, B and F. It will be noted that the gland cell (*GlC*) is much larger than the hypodermal cells (*HypC*) and that an ampulla (*Am*) is present. The secretion collects in the ampulla, passes through the cell wall into the pore canal (*PorC*), then into the reservoir (*Re*), and finally to the outside through the efferent tube (*Ef*). It was not possible to decide definitely whether this tube is open or closed at its inner end; theoretically, it should be closed, but in many cases it seemed to be open. The drawings show it both ways. It is shaped like a finger or a test tube, and its walls are so thin that it really does not matter whether it is closed or open, for in all probability the secretion can pass through its walls by osmosis.

Several years ago the writer (33) collected the literature pertaining to the scent glands or scent-producing organs in insects and classified them on the basis of their distribution. The simplest and commonest type found among beetles and a few other insects is composed of the unicellular glands widely distributed over the body surface. This type is well illustrated on pages 38 to 40 of the above paper, although it is not up to date.

Parts of the body surface of live weevils appear shiny, but not wet, as is often observed in certain other beetles. Several years ago the writer (31) made a careful study of this question in the squash beetle (*Epilachna borealis*). These beetles always appear wet, and the more they are irritated the wetter they become. The secretion, which comes from numerous hypodermal glands widely distributed over the integument, is easily seen on the body surface. It has a bitter taste and emits an odor repugnant to us.

What is the function of this secretion? When it is repellent or distasteful to the insect's enemies, we say that its primary function is for protection and its secondary function probably for recognition. Since the boll weevil does not have a wet body surface, its hypodermal glands certainly do not secrete as copiously as do those in certain other beetles, and in all probability this secretion is not for protection; in which case its primary function must be for recognition. A single boll weevil emits little or no odor noticeable to a person, but when several are confined in a small box or bottle, they emit a characteristic and rather pronounced odor.

Scent glands of this type do not have reservoirs on the body surface to hold the secretion and so prevent it from evaporating too rapidly, but such structures are common in other types. Wherever these glands occur the secretion runs to the exterior and spreads in a film over the chitin and then constantly and perhaps quickly evaporates, giving off its own characteristic odor. However, on the elytra of boll weevils there are peculiar structures called punctures, which might serve as reservoirs for this secretion, provided it spreads sufficiently to reach them. On all parts of the surface (fig. 16, C to F, *po*), except on the wings, where these gland pores occur, they are associated with the hairs, one usually lying at or near the base of each hair (C and E). On the dorsal surface of an elytron there are numerous club-shaped or protective hairs (*j*); numerous gland pores (*po*); and several longitudinal rows or striae of slit-shaped pits, the punctures (*n*). Each puncture consists of an oblong, heavily pigmented border (*l*), a lengthwise slit (E and F, *n*), and a tiny hair (*m*), which lies flatly over and nearly closes the opening of the slit or puncture. The punctures on the right elytron of a male were counted. They lie in 10 rows, ranging from 25 to 39 per row, making a total of 319 for the elytron. What can be the function of these punctures? The writer does not know, unless they serve as reservoirs for some secretion. The minute hair is always present in the same place, and could well serve as a door for these pits.

SENSE ORGANS OF LARVAE OF BOLL WEEVILS

The larvae of boll weevils are white, delicate, and legless grubs, being well protected by the covering of the bolls or squares. Since they spend all their lives inside a dark house, well surrounded by food, the question may properly be asked, "Do they need sense organs?" It is possible that they do not eat all parts of the food surrounding them, in which case they must distinguish the edible from the non-edible by touch, taste, or smell; on the other hand, if they do eat all of the food surrounding them and have sense organs suitable for this purpose, then we conclude that these organs are useless and have only a phylogenetic significance.

OLFACTORY PORES

The olfactory pores on five full-grown larvae were carefully studied. They were found practically constant in position and number, and since those on larva No. 4 were most critically examined and then drawn, they will be described in detail.

No groups of pores were found, but the isolated ones (fig. 11, A to F, *a* to *z*) were usually arranged in pairs; one pore being on the right

side and its mate on the left side of a median line. Ten pairs (*a* to *j*) lie on the head capsule, pore *g* of these being on the microscopic antennae; 3 or 4 pores (*k*) on each mandible; 5 pairs (*l* to *o*) on the labium; 1 pair (*p*) on the mentum; 1 pair (*q*) on the submentum; 1 pair (*r*) on the labrum; 1 pair (*s*) on the clypeus; 8 pores (*t* to *y*) on each maxilla; 2 pairs (*z*) on the second thoracic segment; and 4 pairs (fig. 12, B) on what appears to be the epipharynx.

The external structure (fig. 12, A and B) and internal structure (fig. 12, D to H) of the pores on the larvae are like those already

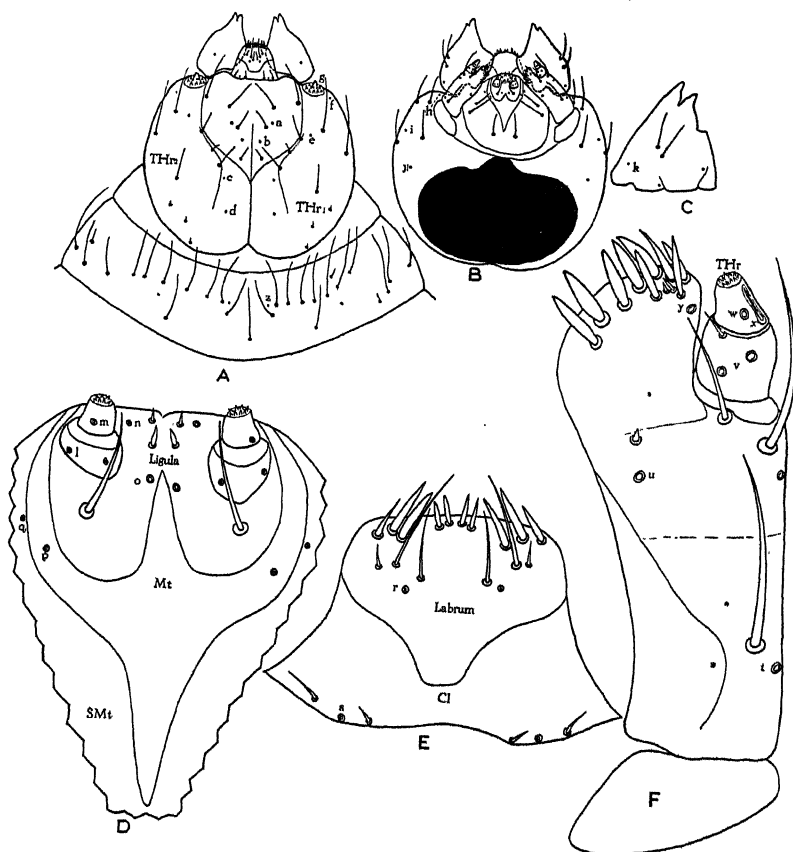


FIG. 11.—Disposition of olfactory pores (*a* to *z*) and hairs on larva of boll weevil. A, dorsal view of head and first two thoracic segments; B, ventral view of head; C, outer surface of right mandible; D, ventral view of labium (Mt), mentum (SMt), and portion of submentum (SMT); E, dorsal view of labrum and clypeus (Cl); and F, ventral view of maxilla. A and B, $\times 31$; C, $\times 52$; D to F, $\times 190$.

described for the adult weevil. On the maxillary palpus (fig. 3, B) of the adult there are five slit-shaped pores, but on that of the larva there is only one (fig. 11, F, and fig. 12, A, *x*).

OLFACTORY PORES OF OTHER LARVAE

No one, except the writer, has apparently identified the olfactory pores as the Hicks' organs in any larva, although it is evident that they have been seen by various systematists, for Schiødte (51, 1872-73)

figured them on the antennae and labial palpi of staphylinid larvae, on the legs of scarabaeid larvae (51, 1874), on the maxillae of lucanid larvae, and on other coleopterous larvae. Böving (2) figured a few of them near the spiracles of a coccinellid larva, and Cotton (6) showed a very few on the mouth parts of different curculionid larvae. Nagel (42) saw two of these pores on the maxillary palpus of a larva of a stonefly (*Perla bicaudata* Panzer); very few on the antennae and labrum of a lepidopterous larva (*Antheraea pernyi* Guér.), and a few widely distributed on the antennae, maxillae, and labium of a coleopterous larva (*Dytiscus marginalis*). Nagel called them "Gruben ohne Kegel" and made sections through them, but did not suggest a function for them, because he failed to understand their internal anatomy. The present writer (35) made a careful study of these pores in 30 species of lepidopterous larvae, and determined experimentally that these larvae respond to chemical stimuli, although no experiments were performed to determine the function of the pores.

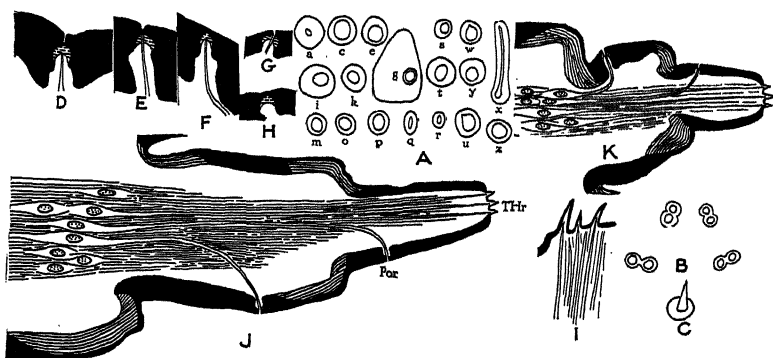


FIG. 12.—A to C, external structure of olfactory pores and tactile hairs of larva of boll weevil. In A the olfactory pores are lettered a to z (with pores b, d, f, h, i, l, n, and r omitted), their position being indicated in Figure 11. A to F: a, c, e, and i, from head capsule; g, antenna with its pore; k, from mandible; m, from labial palpus; o, from ligula; p, from mentum; q, from submentum; r, from labrum; s, from clypeus; t, from stipes; u, from pulvifer; v, w, and x, from palpus; y, from lacinia of maxilla; and z, from second thoracic segment. B, olfactory pores (two near one another) from epipharynx? C, one of smallest tactile hairs (T.Hr., fig. 11, A) on head. Internal anatomy of olfactory pores and innervated hairs of larva of boll weevil. D to H, olfactory pores; D, from labrum; E, from mandible; F, from antenna; G, from maxillary palpus; and H, from labial palpus. I, innervated sense pegs around base of antenna. J and K, from longitudinal sections of maxillary palpus and labial palpus, respectively (mostly diagrammatic), showing innervation of olfactory pores (Por) and sense pegs (Thr). $\times 492$

The writer (34) carefully studied the morphology of the olfactory pores in the larvae of *Cotinis nitida* L. Two types of pores were found, single and compound. The compound were observed only on the terminal segments of the long antennae. Recently the same type has been seen similarly located in the larvae of a May beetle (*Lachnosterna* sp.) and the Japanese beetle (*Popillia japonica* Newm.), two other scarabaeids. The single organs (isolated pores) in *Cotinis* were found on the antennae, mouth parts, head, thorax, and legs. The average total number of pores counted in both types was 1,359. Many drawings of the two types were prepared and published, but photomicrographs, not previously published, are here presented (figs. 13-15) to convince the reader that the sense fibers of these organs actually enter minute pores, called pore apertures. Of course the writer is well aware that such a view is contrary to the accepted belief concerning the presence of pores in the integument of insects; nevertheless he is thoroughly convinced that the ends of these nerves

come in contact with the external air. Just how they end in these pores can not be stated, because they are so small and indistinct that no definite details can be discerned. In the best sections there does not appear to be any membrane, however thin, between the end of the sense fiber and the outside air, although it must be granted that the tip of this fiber is covered by its own cell wall. A careful study of these photomicrographs will show how the two types of pores appear under a high magnification, but particular attention is directed to Figure 13. C shows the complete innervation of an olfactory pore. B is a section of an olfactory pore obtained by using an ordinary photographic plate. Attention is called to the sense fiber (*c*) which passes through the chitinous cone, and stops at A, *a*. A is a section of the same olfactory pore, obtained by using a photographic plate sensitive to yellow. Attention is here called to the pore aperture (*p*) or pit passing from the exterior to the point *a*, where the sense fiber (*c*) enters the pit.

OLFACTORY ORGANS IN MAN AND THE HIGHER ANIMALS

Reference to recent textbooks such, for example, as Howell's "Physiology" (16) and Piersol's "Human Anatomy" (43) will quickly show the reader that the protoplasmic cilia of the olfactory cells in man and the higher animals also come in contact with the outside air. Except in this type of olfactory organ, the microscopic sense cilia are constantly covered with mucus, and in order to produce a sensation of smell the odoriferous particles are first dissolved in this mucus. It is generally believed by the layman that these cilia and their cells are covered by a thin and moist membrane through which the odoriferous air must pass; but the authorities on this subject tell us that wherever the olfactory cells occur in the nasal passages, the epithelium consists of two chief constituents—supporting cells and olfactory cells. The olfactory cells project slightly beyond the general level of the epithelium and each bears six to eight minute stiff cilia, often called olfactory hairs. Mucous glands, lying just beneath the outer or olfactory layer, are very numerous; their orifices are barely distinguishable by the unaided eye; and they keep the outer surface of the membrane well covered with mucus all the time.

The end of each sense fiber in the olfactory pores of insects might be compared to a cilium on an olfactory cell of man. Since the sense cells in insects are modified hypodermal cells, they may still retain a small part of their original secretory use, in which case they would be able to keep the tip ends of their sense fibers sufficiently moist so that the odoriferous particles touching them would be dissolved. If this condition exists, then the olfactory apparatus in insects and the higher animals is very similar in structure, and certainly must be so in function.

TACTILE HAIRS

Since the hairs on the larvae of the boll weevil are comparatively scarce and widely scattered, they were not easily found in sections; consequently many of those called sense hairs and sense bristles in Table 7 have been thus classified merely from their external structure; although the innervation of those at the tips of the maxillary and labial palpi (fig. 11, D, F, and fig. 12, J, THr, and K), and at the base of the antennae (fig. 12, I) has been thoroughly studied.



FIG. 13.—Photomicrographs showing olfactory pores in antenna of larva of *Cotinis nitida*. A, section of olfactory pore obtained by using a photographic plate sensitive to yellow. Attention is called to the pore aperture (*p*) or pit passing from the exterior to the point *a*, which is the end of the sense fiber. It is clear that no membrane separates the end of this fiber from the outside air. B, section of same olfactory pore obtained by using an ordinary photographic plate. Attention is called to the sense fiber (*c*) which passes through the chitinous cone and stops at A, *a*. C, portion of cross section, showing complete innervation of another olfactory pore (*P*); *f*, sense cell, and *g*, branches of nerve. *i*, secondary cuticula. *d*, wall of olfactory pore; *e*, hypodermis; *h*, primary cuticula; and

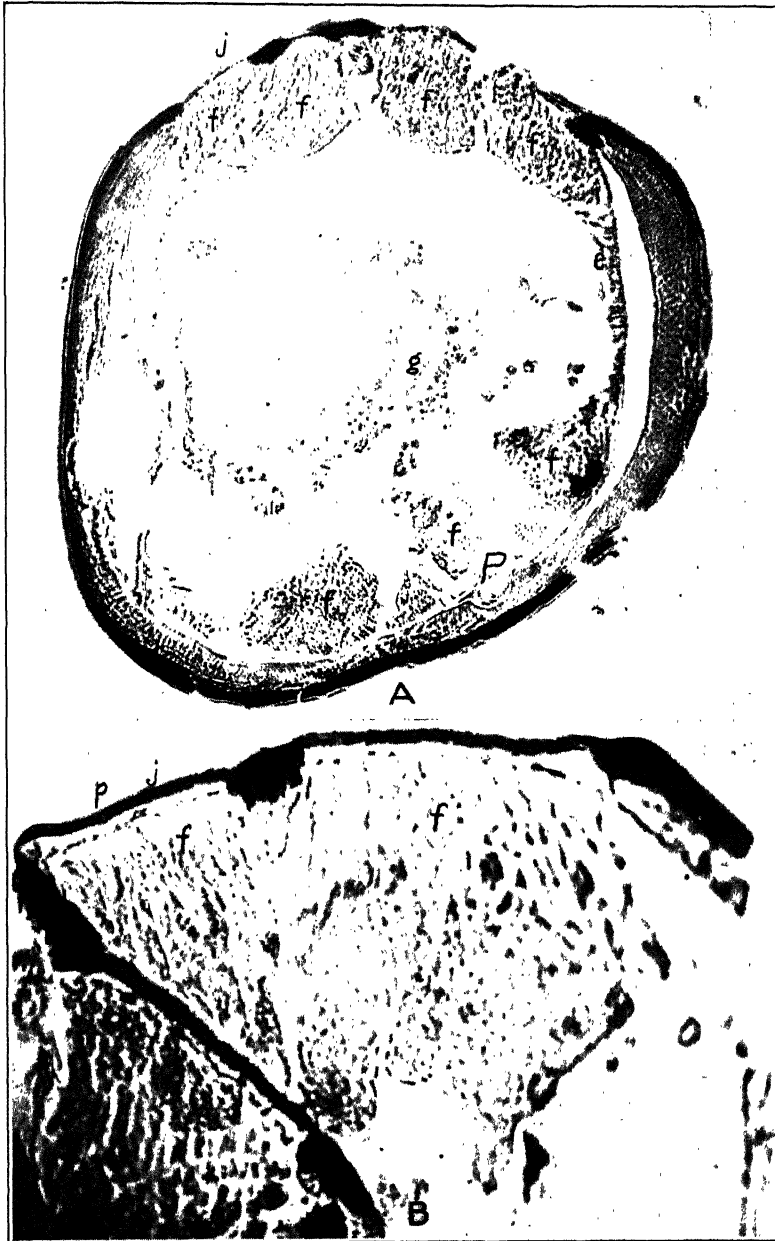


FIG. 14.—Photomicrographs from sections through tips of antennae of *Cotinis nitida*, showing internal anatomy of compound olfactory organs, including *e*, hypodermis; *f*, groups of sense cells; *g*, branches of nerve; *j*, plate over sense cells; *p*, pore through plate; and *P*, a single olfactory pore. A, cross section, and B, portion of longitudinal section

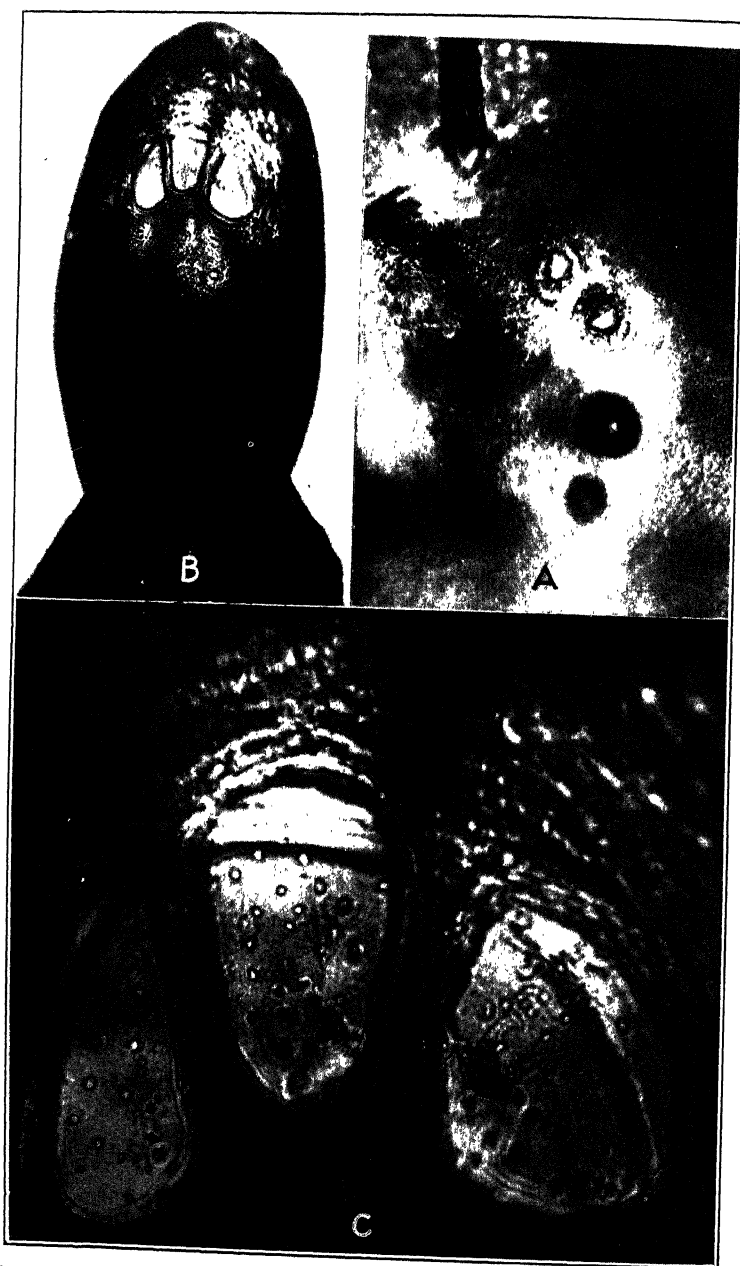


FIG. 15.—Photomicrographs showing disposition of olfactory organs on legs and antennae of *Cotinis nitida*. A, four single olfactory pores (two not in focus) and a hair (at upper left corner) on outer surface of trochanter; B, ventral view of distal end of antenna, showing three compound olfactory organs, and just beneath them four olfactory pores (only one shows plainly); C, a greater magnification of the above three compound olfactory organs. Attention is called to the pore apertures (small circles) in their plates.

Hilton (14), who used the section method for tracing nerves to hairs and also the method of injecting methylene blue just beneath the integument of live larvae, mostly Lepidoptera, summarizes his results as follows:

Lepidopterous larvae are clothed with hollow hairs, each of which is supplied by a bipolar nerve cell, a process of which penetrates a short distance into the hair and probably terminates before reaching the tip. In most species all body hairs are sensory; large hairs are supplied by large bipolar nerve cells, and small ones by smaller bipolar cells. Under the hypodermis of caterpillars there is a system of multipolar cells more or less intimately connected with nerve cells and fibers which stain lighter than the larger nerves and are closer to the hypodermis than the other cells and fibers. Nerves from bipolar sensory nerve cells go to the central nervous system, run to the ganglia, leaving at once to follow on the outside of the connectives cephalad, forming a well-marked sensory tract. Motor nerves—those that go to muscles—seem to come directly from the central cell areas of the ganglia. Almost the only sensory termination of nerves on the body of insects is by means of hairs.

Hilton furthermore states:

In the larvae of May beetles the hairs of the body surface are supplied with bipolar nerve cells.

In the mind of the present writer it is very questionable whether *all* the hairs on any larva are actually sensory.

Zawarzin (60) used a similar method of injecting methylene blue into live larvae of three species of *Aeschna*, and he also used the section method for studying the chitinous apparatus of the sense organs. Very often he had no success with the methylene-blue method, but after much experience he was able to trace nerves to the body hairs.

The present writer, using live larvae of the boll weevil, tried the injection method used by Zawarzin, but had no success in the few preliminary tests conducted, and did not make further attempts because of the lack of live larvae at the time.

TABLE 7.—*Disposition of sense organs on larva of cotton boll weevil*

Divisions	Sense hairs	Sense bristles	Sense pegs	Olfactory pores	Eyes
Head capsule.....	Several.....	Several ?.....	None.....	18.....	Vestigial ocelli.
Antenna.....	None.....	None.....	7.....	1 at base.....	
Mandible.....	do.....	2 ?.....	None.....	3 or 4.....	
Labium.....	2.....	2.....	Several.....	3 on palpus, 4 on ligula.....	
Mentum.....	None.....	None.....	None.....	2.....	
Submentum.....	do.....	do.....	do.....	2.....	
Labrum.....	2.....	4.....	10 ?.....	2.....	
Clypeus.....	2.....	2.....	None.....	2.....	
Maxilla.....	2.....	Several ?.....	Several.....	1 on stipes, 2 on palpi, 1 on labialia, 4 on palpus.....	
Thorax.....	Few on second segment, probably few on other segments.....	Several on second segment, probably several on other segments.....	None.....	4 on second segment.....	
Epipharynx ?.....	None.....	None.....	Few.....	8.....	

OTHER SENSE ORGANS

Besides the olfactory pores and tactile hairs (fig. 11) already described, we should hardly expect to find other sense organs in the larva of the cotton boll weevil; but vestigial ocelli, nevertheless, were found in sections through the head near the antennae. Sphere-

shaped masses of dark-brown granules, the rudiments of the internal anatomy of these eyes, were present, but no convex cornea was visible; however, the chitin above these granular masses was thin and unpigmented, and thus this rudimentary eye might be able to distinguish light from darkness.

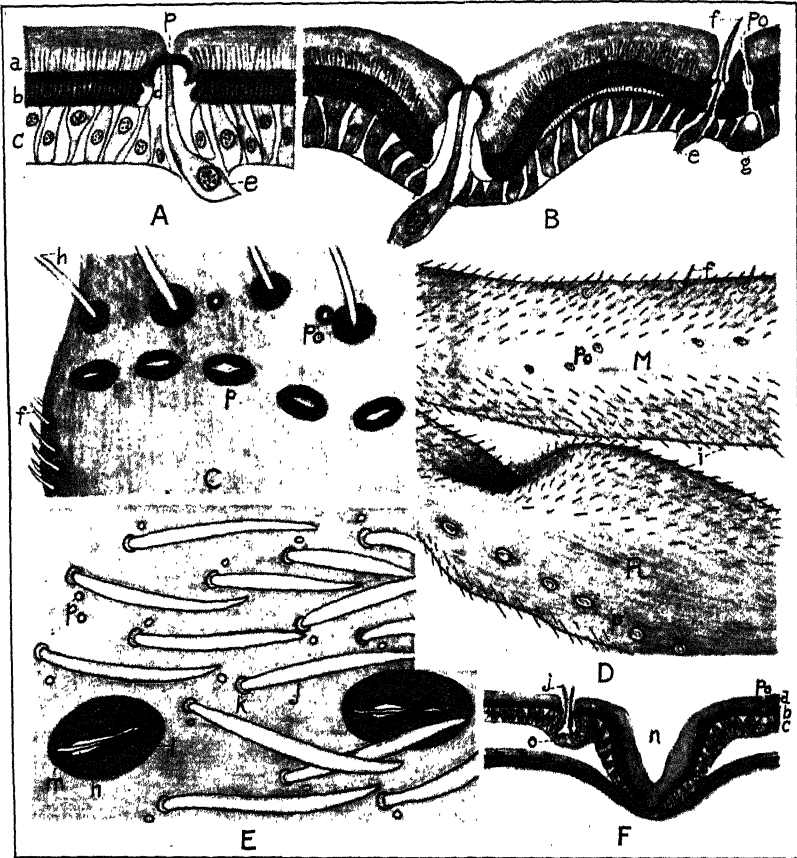


FIG. 16.—Internal and external structure of body wall of boll weevil: All figures are mostly diagrammatic. A, section from tibia; B, section from trochanter; C, superficial view from trochanter of front leg, showing Group 7 of olfactory pores; D, superficial view from wing, showing portions of media (M), radius (R), and six pores of Group 6; E, superficial view from elytron; and F, section passing crosswise through elytral puncture (n).

a, Primary, and b, secondary cuticula; c, hypodermis; d, chitinous cone; e, sense cell; f, tactile hair (No. 1); g, gland cell; h, noninnervated hair on leg; i, prickle or noninnervated pseudohair on wing; j, large club-shaped, noninnervated hair on elytron; k, hair socket or alveolus; l, border of puncture; m, noninnervated hair lying over opening (n) of elytral puncture; o, trichogen cell forming club-shaped hair (j); p, olfactory pore; and po, pore of gland. A and B, $\times 714$; C to F, $\times 271$.

No attempt was made to find chordotonal organs in the larva. They may be present, for Comstock (5, p. 148) says:

Chordotonal organs have been observed in so many larvae that we may infer that they are commonly present in larvae. These organs are very simple compared with those of certain adult insects.

Credit is due Schiødte (51, 1869) for describing and figuring chordotonal organs for the first time. He found them on the thoracic segments of buprestid larvae. Graber (10) described chordotonal

organs in the larvae of *Dytiscus*, and Hess (12) has given us a good description of them in cerambycid larvae. In 1874 Schiødte (51, p. 88) gave an account of the stridulating organs in the larvae of certain Scarabaeidae and Lucanidae.

Larvae, like adult insects, certainly have general senses, but nothing definite is known about them. The subhypodermal nerve plexus, described by Hilton (14) and several earlier writers, may serve as receptors for temperature and humidity.

HOW PLANTS ATTRACT INSECTS BY SMELL

It was assumed in the introduction that cotton plants emit odors which attract boll weevils, but so far no proof in support of this assumption has been given. Although the writer has not been in a position to work extensively with boll weevils and cotton plants, he has nevertheless carried on the same line of work using other insects and their host plants. Only a very brief summary of the results of these experiments can be given here.

As flowers are generally more or less fragrant, so the foliage of most plants also emits odors. These odors are usually weak, judged by the human olfactory sense, but to the insect which feeds upon the foliage such may not be the case. Of course, it was impossible to test the response of the insect to the odor of its host plant in the field, because the interfering factors could not be controlled. Consequently it was necessary to develop a special apparatus to be used in the laboratory. A full description of this apparatus, called an insect "olfactometer" and already mentioned under "olfactory experiments," is now in print (38).

The name "olfactometer" means an olfactory measuring apparatus. The one devised has been used to measure or record the responses of insects to attractants and repellents. Briefly stated, it consists of a specially constructed Y-tube, through which insects pass from a dark chamber, being attracted by a light suspended near the free ends of the forks of this tube. The dark chamber is attached to the base of the Y-tube, and a suction apparatus to draw odors through the forks is attached at the point where the base and forks unite. The insects are attracted equally toward the entrances of the forks by the light stimulus, but when ready to enter these forks they are influenced unequally by the odors drawn through the forks, one fork serving as an attractant or repellent side, and the other fork as the control side.

The whole apparatus is so constructed and manipulated that the interfering factors are practically controlled, leaving only the olfactory responses to be recorded.

Only the results obtained with the Colorado potato beetle (*Leptinotarsa decemlineata* Say) will be given here to show that plants attract insects by the odors which they emit. A small potted potato plant was placed in a special chamber of the olfactometer, and a gentle current of air was drawn over it into one fork of the Y-tube by means of the suction apparatus. This supposedly scented air could not be smelled by the writer, but the potato beetles responded to it readily. This experiment on various dates and under different conditions was repeated 24 times, 4 individual tests being considered as one experiment. Each of these 25 experiments plainly showed that the beetles had responded to the odors or emanations from the living potato plants. On an average, 62.7 per cent of them went toward these

odors, while the other 37.3 per cent went (perhaps accidentally) into the control fork. The highest percentage obtained was 76.7 toward the plant odors. Potato beetles, when potato plants are scarce or entirely wanting, feed on the horse nettle, jimson weed, and tomato plant. Experiments similar to the above were conducted in which these plants were used, but as a rule the beetles did not seem to like the odors from these three species. They often failed to respond, and not once did their response give a high percentage of attractiveness.

Eighty experiments were conducted, using the water extracts (diluted juices) and steam distillates of the potato tuber and foliage of the following plants: Potato, jimson weed, horse nettle, tomato, and henbane. In these experiments air was bubbled through the liquids and then drawn into one fork of the Y-tube. In the other or control fork air was bubbled through distilled water. To the odors from the water extracts of potato tubers and potato foliage the beetles always responded, except once, but not as strongly as to the odors from the living potato plants. To the odors from the extracts of the other plants mentioned the beetles usually responded, and more strongly than they had to the odors from the living plants of the same species. To the odors from the steam distillates the beetles generally responded, although many times repellently. The odors from the jimson weed distillate remained repellent throughout the tests; the odors from the other distillates were usually repellent for some time, but later became attractive. The odors from the distillates of the potato tuber, horse nettle, and tomato were only slightly attractive, but those from the potato foliage and henbane were usually strongly attractive. It seems that the steam removed something from these plants which was repellent to the beetles for a time, but later, when this apparently repellent substance had been either changed or volatilized by bubbling air through the liquids, the odors became attractive. When the odors from all these distillates, except from jimson weed, had become attractive to the insects, the liquids gave off a common odor, judged by the nose of the writer. This odor closely resembled that from boiling potatoes or from the juice of freshly cut potatoes, and might be called a potato odor. Since all of these plants belong to the potato family (*Solanaceae*), it would be interesting to know whether they possess a particular constituent or constituents which emit a common odor, as indicated by the above experiments.

Assuming that the cotton plant emits some volatile and odorous substance which attracts boll weevils from a considerable distance, Power and Chesnut (44, 45), of the Bureau of Chemistry, undertook to isolate this substance. Among the 12 individual substances found by them in the concentrated distillate, which included all the odorous and volatile constituents in the cotton plant, 3 are of particular interest for chemotropic tests. These are the so-called essential oil and 2 basic substances, ammonia and trimethylamine. The percentage of oil isolated is so very small that these writers believe it can not have any significance in attracting boll weevils, and, furthermore, despite the fact that more ammonia was found than trimethylamine, they conclude that if the cotton plant really possesses an attractive odorous constituent, it is in all probability the trimethylamine. If so, the difficult task for the entomologist is to use the substance properly. Of all substances having powerful and persistent odors, trimethylamine is certainly one of them, as illustrated by

the following. Before using a small bottleful of this material, prepared by Power and Chesnut, the writer kept it well stoppered for a few days in a drawer with a small dictionary and some stationery. Both became permeated with the odor, and for weeks afterwards smelled strongly of it. The dictionary still emits a faint odor after a period of two years.

The writer tested the sample of trimethylamine mentioned above in only a preliminary way, using a comparatively few old weevils which had come to the cotton plants from their hibernating places, and the most that he can say in its favor is that it seemed to attract the weevils slightly under certain conditions.

The writer entertains a serious doubt whether it is possible to reproduce accurately the odor or odors which emanate from a plant merely by using the constituents derived from the plant by chemical means. It seems that the most that may be hoped for is an imitation closely resembling the plant odor and even with this it may be difficult and sometimes impossible to deceive the insects. This whole subject is closely related and perhaps analagous to the making of perfumes for the use of the human species. Expert perfumers admit that, after all their long experience in trying to reproduce exactly the odors found in living flowers, the best they have been able to do so far is to imitate closely the natural odors. In many cases the layman can not tell these artificial odors from the natural ones, but the expert perfume tester can readily distinguish them. Insects, which evidently have a keener sense of smell than human beings, should be able to distinguish these differences even more readily.

To find a substance, preferably a sweet one, which would attract boll weevils equally as well as do cotton squares (flower buds), many experiments were conducted in the laboratory and cotton field, using saccharine, sugar, ice-cream powder, a sweetened proprietary calcium arsenate, honey, and three brands of molasses (karo, koo-koo, and blackstrap). The honey and ice-cream powder seemed to attract the weevils slightly, but there was no indication that any of these eight substances can be of practical importance in the control of these insects. Similar results have been obtained by other writers who have tried to attract boll weevils by the use of sweetened baits.

SUMMARY OF SENSES OF COTTON BOLL WEEVIL

Since the sense of smell in boll weevils is believed to be the primary one which aids them in locating cotton plants, the two kinds of so-called olfactory organs have been thoroughly studied and described. The organs, called olfactory pores by the writer, are common to both the adult and larva; but the other so-called olfactory organs, which are nothing more than ordinary innervated hairs, are common only to the antennae of the adult, although similar innervated hairs are also found on other parts of both adult and larva. In the adult the olfactory pores were found on the head capsules, legs, elytra, wings, mouth parts, and at the base of the antennae; in the larva, on the head capsule, base of antennae, mouth parts, clypeus, and second thoracic segment. The individual and sexual variations found in the pores of each of five males and five females were small, although the females have 13.7 per cent more pores than have the males. The anatomy of these pores is like that described for many other beetles by the writer (30), except that some

of them are slit shaped, closely resembling those in spiders. These are found on the maxillary palpi of both adult and larva and on the legs of the adult.

The antennae of boll weevils have four types of sense organs, as follows: Many innervated hairs, chiefly on the club; three or four olfactory pores at the base of each antenna; and two so-called auditory organs in the second segment. Not one of these organs, except the olfactory pores (fig. 13), is suited anatomically to receive olfactory stimuli. The hairs are all long, thick-walled throughout their full length, and consequently odors can not pass through them. The so-called auditory organs are totally internal, having no outside connections whatever, so that they certainly can not receive olfactory stimuli.

Innervated hairs were found widely distributed over the surface of the boll weevil, being present on the head capsule, antennae, mouth parts, thorax, legs, wings, abdomen, and genitalia. According to their structure they seem to be fitted only for tactile organs, and, judging from their number and distribution, the sense of touch must be highly developed. When the insect is once upon its proper food and ready to deposit eggs, the tactile sense may be more important than the olfactory sense. The numerous sensitive hairs on the antennae, mouth parts, ovipositor, penis, and elsewhere must certainly render great aid in the various activities of the boll weevil.

No organs were found which can really be called taste organs, although some of the smaller innervated hairs on the mouth parts of both adult and larva would be designated taste organs by certain writers. The present writer believes that the senses of smell and taste in insects are inseparable, but since insects in many cases must first "taste" certain foods before they can discriminate between them, this combined sense may be comparable to ours when we smell flavors.

Three so-called auditory organs were found—two in the pedicel or second antennal segment and one in the base of each wing. We know nothing about the function of these organs, and, indeed, it is difficult to imagine what their offices might be, unless they receive some kinds of waves which we can not detect, such as some of the inaudible sound waves, or even radio waves; but the writer has been informed that radio waves can not be considered in this connection.

Judging from the structure of the compound eyes, the visual field of the boll weevil is small and its image of objects is neither sharp nor distinct, indicating that it can not by sight alone distinguish from a distance cotton plants from certain other plants, or in all probability even the various parts of a cotton plant when it is near them.

As a corollary to the sense organs, the scent-producing organs, sometimes called scent glands or recognition glands, were also studied. These are minute, unicellular, hypodermal glands, and are widely distributed over the surface of the insect. Their secretion comes to the surface, spreads out over the integument, and, besides keeping the body moist, is also supposed to give off an odor which probably serves as a recognition odor among the beetles themselves.

The reader will have noted that the olfactory sense has been greatly emphasized, perhaps too much, but in the present state of our knowledge there seems to be no other tangible sense or senses which serve quite so well to explain how boll weevils find cotton plants from

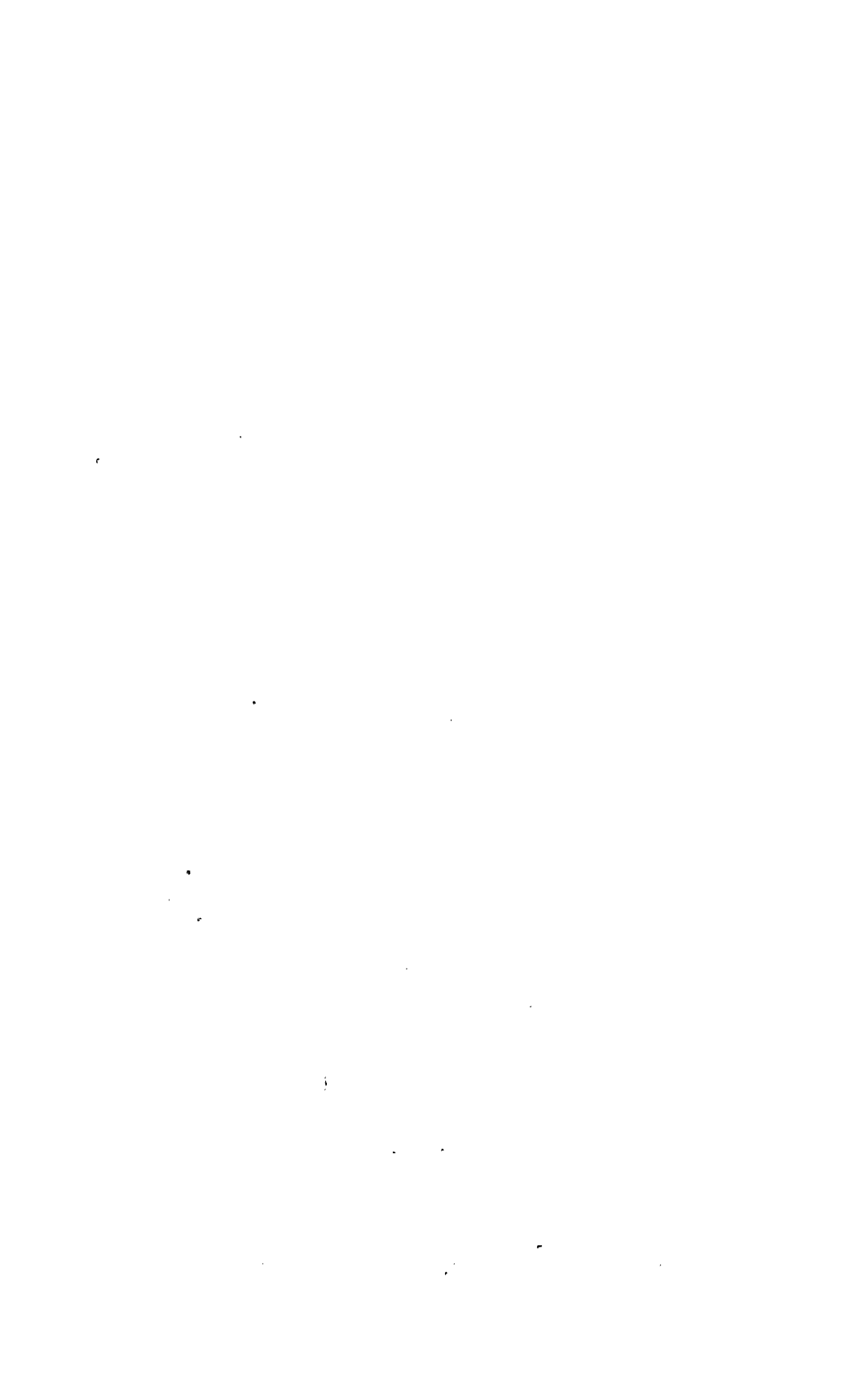
a distance. When they have once found their food or a place to deposit eggs, we can easily think of two or more senses being employed, in their activities thereafter, touch, smell, and "taste" particularly being used during feeding and egg laying, as has recently been pointed out by Richardson (49), who reviewed the literature pertaining to the oviposition response of insects.

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EFFORTS TO DETERMINE THE MEANS BY WHICH THE COTTON-WILT FUNGUS, *FUSARIUM VASINFECTIONUM*, INDUCES WILTING¹

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HISTORICAL REVIEW

In 1899 Smith (25)² suggested that the wilting of cotton, cowpea, and watermelon plants, which were infected with closely related fungi of the genus *Fusarium*, was due to a mechanical plugging up of the vascular system. He was able to find considerable mycelium within the water-conducting tubes, and assumed that the amount present was sufficient to act as an obstruction to the passage of liquid upwards, thus causing the condition known as "wilt." His explanation has for many years been the one commonly accepted by writers of various well-known texts, as well as by authors of special bulletins dealing with cotton wilt, including Duggar (7), Stevens (26), and Orton (20, 21, 22).

As this theory has within recent years been questioned in a number of comparable diseases caused by species of *Fusarium*, such as those causing potato wilt (3; 5, p. 270; 11; 12) and tomato wilt (9, p. 75), as well as wilts induced by other fungi and bacteria (2, 8, 23), it seemed desirable to ascertain the mode of action of *F. vasinfectum* in its method of inducing cotton plants to wilt. It appeared especially worth while to undertake such a study, in view of the lack of wilting noticed in certain infested fields at times, which in other seasons showed a high wilt percentage. Was this explainable on the basis of difference in environmental conditions, such as soil or air temperatures, soil moisture, etc., or was it to be regarded as a difference in the degree of plugging up of the vascular systems resulting from a difference in the amount of growth of the fungus? If wilting is simply a response to a mechanical obstruction, then it is quite conceivable that any condition which would favor the growth of the fungus, irrespective of its effect on the host, should make for a wilting of the plant; and, on the other hand, any condition which would be unfavorable to the growth of the fungus should make for wilt-free plants.

One of the first to experiment on the factors involved in the wilting of a plant attacked by a microorganism was Hutchinson (14) of India. In 1913, working on a disease of tobacco which he considered to be caused by *Bacillus solanacearum*, he fed plants a substance which had been precipitated by alcohol from pure bouillon cultures of the pathogene. The plants which obtained this substance developed wilt, while those fed with a boiled solution of the same substance did

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² Reference is made by number (italic) to "Literature cited," p. 1161.

not wilt. He considers the wilting as due to the interference of osmotic pressure consequent on protoplasmic intoxication, and in the advanced stages the water supply is interfered with by the formation and acceleration of gum masses in vessels. The actual factor which brought about these conditions is a thermolabile, toxic substance.

Coons (5, p. 270) reporting on the work of Goss in 1916 in a preliminary fashion, calls attention to the wilting of Irish potato plants when placed in a filtrate representing a synthetic solution upon which a species of *Fusarium* had been grown. Control plants placed in an uninoculated medium remained healthy. It was concluded that wilting was due to poisons generated by the fungus.

Haskell (12) in 1919 came to a similar conclusion concerning wilt of Irish potatoes caused by *Fusarium oxysporum*. He injected solutions of oxalic and salicylic acids, as well as liquid culture media upon which the fungus had been grown, and obtained a browning and necrosis of the vascular systems with all. By making microscopic observations of stems of infected plants, he "found no instance of a stoppage of the trachea sufficient to shut off the passage of sap."

In 1919 Bisby (3) reported filtering several species of fungi from the liquid nutrient media upon which they had grown, and placing leaves of different plants into the filtrates. The fungi used were: *Fusarium oxysporum*, *F. discolor sulphureum*, *Fusarium* from pea, *Rhizoctonia*, and *Penicillium*. Wilting occurred within a few hours with some extracts, a fact which he feels is not to be explained by changes in acidity. Excised leaves placed in uninoculated media and in water did not wilt until considerable time had elapsed. The injurious effect was also noted after boiling the filtrates and after diluting them considerably. He concludes that the wilting is not due to some poison which is specifically active against certain plants, since "potato leaves wilt as readily in old *Rhizopus* solutions as in solutions in which *F. oxysporum* had grown."

Brandes (4) in 1919 found that cultures of *Fusarium cubense* grown in Richards' solution when filtered free from the fungus produced a marked wilting of buckwheat and bean plants, as well as banana leaves. He obtained the same results, using Urechinsky's solution as a medium, and concluded that in the disease known as banana wilt, wilting is not due to plugging of the vessels by mycelium, but is probably the result of toxic excretions by the fungus.

Young and Bennett in 1920 (28) made a further effort to determine the method by which *Fusarium oxysporum* induces wilting of potato plants. They found that filtrates of this fungus growing upon Richards' solution were increasingly toxic to potato plants as the age of the cultures increased up to the fortieth day. Furthermore, with this increase in toxicity there is noted, after a 10-day period, a gradual decrease in the hydrogen-ion concentration, reaching P_H 7.4 on the fortieth day from an initial reaction of P_H 5.0. But upon uninoculated solution adjusted to P_H 7.4, there is no toxicity noted when cut stems are placed in it. From this it is concluded that the change in reaction, while not the primary cause of wilting, indicates the presence of a compound with a slightly alkaline reaction. Autoclaving or boiling did not alter the toxicity of this substance.

In 1921 Ajrekar and Bal (1) of India, working on cotton wilt and using Hutchinson's (14) method, previously mentioned, were unable to obtain wilting of cotton plants by means of the alcoholic extract. They do not, however, conclude from their very meager tests (only two plants were used) that no toxins are produced by the fungus; on the contrary, when they sectioned diseased plants and examined them microscopically, the number of vessels which were not occupied by the fungus was so great in comparison with the number occupied that they suspected the action of a toxic substance rather than a mechanical plugging up by fungal mycelium.

Bewley (2) in 1922 found that the liquid cultures in which *Verticillium albo-atrum* was grown (the fungus causing "sleepy disease" of tomato) contained a substance capable of causing wilt when filtered free from the fungus. He attempted to isolate the enzymes produced by the fungus during growth and decided that no endoenzymes capable of causing wilt were present. He concluded that the wilting was caused by an exoenzyme which he precipitated with alcohol, and which he found to be greatly reduced in activity by heating to 100° C.

Picado (23) in 1923 attempted to determine the nature of the substances secreted or excreted by parasitic fungi in which the host tissues are either injured or killed at some distance from the point of attack. He worked with *Verticillium albo-atrum*, *V. dubois*, both parasites of the potato; *Fusarium solani* from the melon; *F. cubense*, the pathogene of banana wilt; and with a saprophytic *Penicillium*. The results indicated that the withering of the plants experimented upon, the browning of their vessels, and the destruction of their tissues were not all produced by the same causes, though in each instance evidence was seen of the action of substances having toxic properties, such action resulting in the symptoms produced in the different diseases. Picado draws a distinction between true toxins or enzymes and substances having poisonous properties. After extracting the albuminoids, enzymes, and toxins from the mycelium with alcohol and concentrating the liquid from the crushed mycelium, he obtained a waxy, brownish residue. This proved to be thermostable and very toxic to plants. The heated extracts were considerably more potent in producing wilt than the endoenzymes and endotoxins. Picado's conclusion is that the poisons produced by these fungi are not true toxins in the sense of toxins against which immunization is possible. They may be compared in action to poisons found in some mushrooms such as "Amanita-toxin," and he suggests that the action at a distance of certain parasitic fungi may be due to a chronic poisoning of the plant.

In 1923 Fahmy (8) described his efforts to determine the cause of wilting brought about by *Fusarium solani*. The fungus was grown in pure culture in Richards' solution, and then filtered under sterile conditions. He found that the unboiled as well as the boiled filtrate possessed marked toxic properties, and he determined the presence in the filtrate of ammonia as well as oxalic acid. But the amounts present did not seem to him to be sufficient to account for the wilting. He also found that the substance responsible for wilting was thermostable and nonvolatile.

Goss (11) in 1924 concluded upon the examination of infected potato plants that the vascular elements were not blocked by the growth of the fungus, *Fusarium eumartii*, and that the tissues were often killed in advance of the organism. He grew the fungus on a slightly modified Richards' solution, and after filtering he found the filtrate capable of wilting plants in a relatively short time. The boiled extract, however, produced no wilting. In contrast to this, filtrates of another wilt-inducing fungus, *Fusarium oxysporum*, remained toxic after boiling.

In the biennial report of the director of the Kansas Agricultural Experiment Station, which appeared in 1924 (9, p. 75), it is recorded that *Fusarium lycopersici* has been found to secrete an enzyme which, when precipitated, dried, and redissolved in distilled water, causes a rapid wilting of young plants when their cut stems are immersed in the solution. Boiling destroys the toxicity of the enzyme. Both the residue and the dialyzate of nutrient solutions upon which the fungus was grown produced wilting of young plants. From this it is concluded that two toxic substances exist, one a colloid and the other a crystalloid.

The recent work of Johnson and Murwin (15) on the toxic substance produced by the bacterium causing wildfire of tobacco is of interest. They noticed marked yellowish halos and chlorosis of bud leaves in the absence of organisms or with a few present. They concluded that a soluble, toxic substance is formed by the organism. When it was cultured on potato agar and a suspension made of the pathogene in water, which was filtered through a Berkefeld filter, the application of the cell-free filtrate on tobacco leaves resulted in the production of typical halos.

Very recently Jochinai (27) concluded that the rapid wilting of flax plants attacked by *Fusarium lini* was due to the obstruction of the xylem consequent upon the production of gas, while the poisoning of the cells was caused by alkalinity produced by the fungus. His conclusions are based entirely upon *in vitro* studies of fungal cultures and no effort was made to test these theories in healthy host plants.

These references, and others not cited, all indicate that in a number of diseases, many of them comparable to the wilt of cotton in symptoms as well as in causal agent, the theory of a mechanical plugging by the fungus causing wilt has been discarded and in place of it there is proposed the action of one or more toxic substances given off by the various fungi. These toxic substances are directly or indirectly responsible for wilting and other pathological phenomena. But it is quite obvious that in very few instances has a careful study been made of the nature of the poisonous principles produced by the fungi. It has also been revealed that an attempt to prove the production of a toxic substance by the cotton wilt fungus resulted in failure.

EXPERIMENTS TO DETERMINE THE ACTION OF FILTRATES ON COTTON PLANTS

From the literature cited it appears that the common method used in proving the presence or absence of toxic substances is to grow the pathogene on some nutrient medium—frequently Richards'

solution is utilized—filter the organism from the medium, and place the plants, usually with their roots cut off, into the filtrate. This method has been utilized by the writer (its adequacy will be discussed later). Table 1 presents in brief the number of such tests, and the results obtained. Unless otherwise noted, the plants consisted of vigorously growing seedlings which were carefully removed from the pots, then placed in water and the roots cut off under the water. The cultures of the fungi were grown at room temperature for two to three weeks in Erlenmeyer flasks containing about 300 c. c. of medium.

TABLE 1.—Action of various filtrates on cotton plants

Experiment No.	Date	Fungus	Strain	Medium	Type of filtration	Number of plants treated	Number of plants wilted	Number of plants free from wilt	Length of time for wilt to appear
1a	1925 Feb. 16	F. vasinfectum	1924 Terrace	Nutrient broth	Fine filter paper	12	0	12	18 hours. ^a
1b	do	do	do	Richards' solution	do	12	12	12	18
1c	do	do	do	Uninoculated Richards' solution	do	12	12	12	20 hours. ^b
2a	1925 Feb. 24	F. lycopersici	1924 Terrace	Richards' solution	Fine filter paper	12	14	0	15 hours.
2b	do	do	do	do	do	6	6	0	Do.
2c	do	do	do	do	do	6	5	1	Do.
2d	do	do	do	do	do	12	0	12	Do.
3a	1925 Feb. 25	F. vasinfectum	1924 Terrace	Uninoculated Richards' solution	Fine filter paper	3	2	1	24 hours.
3b	do	do	do	Richards' solution, acidulated	do	6	6	0	Do.
3c	do	do	do	Richards' solution	do	6	6	0	Do.
4a	1925 Mar. 3	do	do	Ushinsky's solution	Porcelain filter	8	0	8	Do.
4b	do	do	do	Uninoculated Ushinsky's solution	Berkfeld N filter	8	0	8	Do.
5a	1925 Mar. 16	F. vasinfectum	1924 Acala	Richards' solution	Berkfeld N filter	3	3	0	10 minutes.
5b	do	do	do	Tap water	do	3	0	3	Do.
5c	1925 Mar. 27	F. vasinfectum	1924 Acala	Richards' solution	Berkfeld N filter	4	4	0	20 hours.
5d	do	do	do	Uninoculated Richards' solution	do	4	0	4	Do.
6a	do	do	do	Richards' solution diluted with equal quantity of distilled H ₂ O.	Berkfeld N filter	4	4	0	20 hours.
6b	do	do	do	Uninoculated Richards' solution diluted with equal quantity of distilled H ₂ O.	do	4	0	4	Do.
6c	do	do	do	Richards' solution diluted with equal quantity of distilled H ₂ O.	do	4	0	4	Do.
6d	do	do	do	Uninoculated Richards' solution diluted as in 6c.	do	4	0	4	Do.
6e	do	do	do	Richards' solution diluted with distilled H ₂ O, 1:2.	do	3	0	3	Do.
7a	do	do	do	Richards' solution containing glucose.	Berkfeld N filter	5	5	0	20 hours.
7b	do	do	do	Uninoculated Richards' solution.	do	5	5	0	Do.
7c	do	do	do	Richards' solution diluted with equal quantity H ₂ O.	Berkfeld N filter	5	5	0	Do.
7d	do	do	do	Uninoculated Richards' solution diluted as in 7c.	do	5	5	0	Do.
8a	1925 Mar. 31	F. vasinfectum	1924 Acala	Richards' solution	Berkfeld N filter	3	3	0	5 hours.
8b	do	do	do	do	Filtrate of 8a diluted with equal quantity H ₂ O.	3	3	0	Do.
8c	do	do	do	do	Filtrate of 8a diluted with twice its volume of H ₂ O.	3	0	3	Do.
8d	do	do	do	Uninoculated Richards' solution.	do	3	0	3	Do.

8e	do	8d solution diluted with equal volume H ₂ O	3	0	3
8f	do	8d solution diluted with twice its volume of H ₂ O	3	0	3
9a	F. vasinfectum	1924 Acela Richards' solution distilled at 100° C.	4	4	0 24 hours.
9b	do	do Richards' solution distilled at 110-120° C.	4	0	4
9c	do	do Residue of distillation in 9a and 9b, diluted 1 to 5 parts H ₂ O.	4	4	0 8 hours.
9d	do	do Same as 9c, diluted 1 to 20 parts H ₂ O.	3	3	0 10 hours.
9e	do	do Same as 9c, diluted with H ₂ O to make a 2 per cent solution.	5	5	0 24 hours, slight wilting.
9f	do	do Same as 9c, diluted to make a 1 per cent solution.	4	4	0 48 hours, slight wilting.
10a	June 24	do Single spore	9	5	5 hours.
10b	do	do Richards' solution (boiled)	9	5	Do.
10c	do	do Uninoculated Richards' solution.	9	0	9
11a	1926 Jan. 11	do F. vasinfectum	3	3	0 5 hours.
11b	do	do F. tracheipilum	3	3	0 Do.
11c	do	do Uninoculated Richards' solution.	3	0	3
11d	do	do Tap water	3	0	3
12a	Feb. 5	do F. vasinfectum	4	4	0 16 hours.
12b	do	do do	4	4	0 Do.
12c	do	do do	4	4	0 Do.
12d	do	do do	4	4	0 Do.
12e	do	do do	4	0	4
12f	do	do do	4	0	4

^a Plants kept in the shade, temperature about 22° C.

^b Slight wilting.

^c After standing in the filtrate for 20 hours the plants of experiments 1b, 1c, and 1d were placed in tap water to prevent the action of any contaminating organisms that might appear in the filtrate.

EXPERIMENTS 1A TO 1D

At the end of a week the plants originally placed in uninoculated Richards' solution appeared perfectly healthy; those in the *Fusarium vasinfectum* filtrate were dead; those in *F. lycopersici* filtrate, with the exception of two plants that appeared normal, showed varying degrees of withering and flagging. On the upper parts of the stems of plants treated with *F. vasinfectum* filtrate, profuse growth of a *Fusarium* developed, but the plants subjected to the *F. lycopersici* filtrate showed no such growths. As will be shown later, filtering through fine filter paper does not remove all of the fungus, and a probable explanation for the lack of fungal development on wilted stems of plants treated with *F. lycopersici* filtrate is that that fungus will not

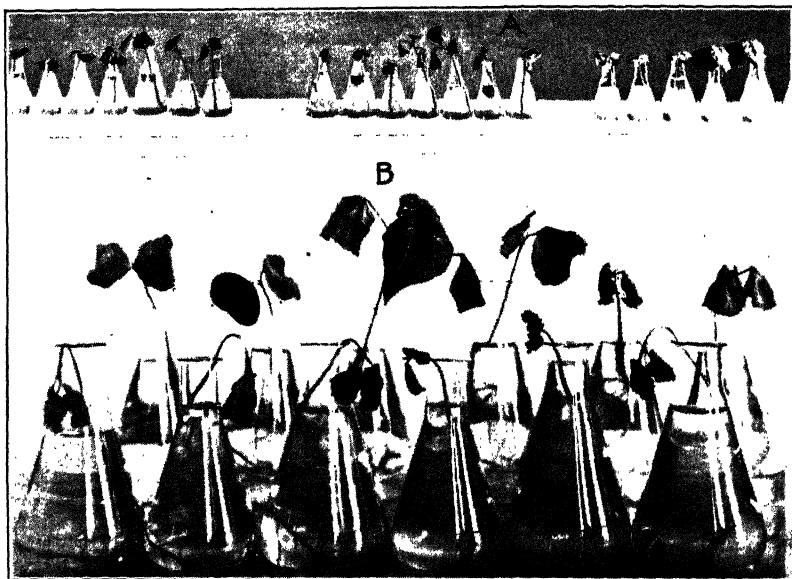


FIG. 1.—A, comparison of the action of a filtrate of *Fusarium vasinfectum* (the 7 plants in the center), a filtrate of *F. lycopersici* (the 7 plants on the left), and uninoculated Richards' solution (the 6 plants on the right). Photographed at the end of 24 hours' treatment. B, comparison of the action of a filtrate which had passed through a bacteriological porcelain filter (top row) and a filtrate which had passed through a fine grade of filter paper (bottom row). Photographed at the end of 24 hours' treatment. In both cases the plants had been carefully removed from the soil and the roots immersed in the filtrate

grow on cotton, although the filtrate contains ingredients which are poisonous. The interiors of wilted plants all showed brownish discoloration of the xylem elements, while the unwilted control plants remained normal.

One of the interesting facts noted in this series of experiments is the difference between the action of the nutrient-broth filtrate and Richards' solution (see fig. 1), both representing transfers from the same fungal culture, and both of exactly the same age. The fact that the broth filtrate possessed no toxic properties, while Richards' solution proved markedly toxic, may explain the reason for the failure of Ajrekar and Bal (1) to obtain toxic action of the cotton-wilt fungus when grown in peptone bouillon. It also brings to mind the marked

difference in physiological processes which are apt to appear with the use of different types of media, or under other differences in environmental conditions. For example, it is well known that in the presence of certain organic nitrogen compounds the metabolic products attending the growth of numerous microorganisms are apt to be different from those found when inorganic nitrogen is supplied. Again, in the presence of certain carbohydrates, acids and gas are formed, while in the absence of these no acid or gas formation takes place. It opens up the whole question as to the significance to be attached to findings of toxic properties with any medium which does not closely approach the chemical and physical make-up of the natural host.

EXPERIMENTS 2A TO 2D

The porcelain filter used in experiments 2a to 2d was one which did not permit the passage of a 10 per cent solution of defibrinated ox-blood hemoglobin. It had been previously autoclaved for 30 minutes at 15 pounds pressure, and the whole process of filtering carried on under aseptic conditions. Platings made of this filtrate remained sterile.

The one plant which had not wilted in the 15-hour period in experiment 2b gradually succumbed, so that at the end of 36 hours all of the plants treated with the filtrate which had passed through the porcelain filter appeared just as withered and sickly as those which had been placed in the filtrate of the filter paper. Platings made of the latter showed that a good many viable spores of the fungus had passed through the pores of the paper. These made typical growth, with the pinkish color characteristic of *Fusarium vasinfectum* when grown on potato-glucose agar.

As the uninoculated Richards' solution tested P_H 4.2, while the filtrate of the 3-weeks-old culture tested P_H 6.6, it seemed worthwhile to determine if the change in acidity had any influence on the toxic properties of the filtrate. The filtrate in experiment 2d was acidulated with hydrochloric acid until it tested P_H 4.4. As far as this limited test goes, it appears that the change in hydrogen-ion concentration in itself was not responsible for the poisonous nature of the filtrate.

EXPERIMENTS 3A AND 3B

Experiments 3a and 3b were designed primarily to test the action of the filtrates upon uncut stems. The plants were carefully removed from the pots of soil in such a manner as to disturb the root systems as little as possible. The roots were washed free of soil and then placed in the filtrates. The results show that the toxic substance can be absorbed by the roots and produce typical wilt symptoms. May not this fact explain why in a given field certain plants exhibit at times a stunted and partially wilted effect, without any evidence of infection, while other plants readily yield cultures of the cotton-wilt fungus?

One fact observed in this experiment was the more rapid and complete wilting of the plants placed in the filter-paper filtrate, as compared with those placed in the filtrate which had passed through the porcelain cylinder. This had also been noticed in the previous experiments. It has already been noted that solutions which passed through

fine filter paper still harbored the fungus. This unquestionably would also be true of cotton filters, and as a number of investigators whose work has been previously reported say nothing of the use of bacteriological filters, it opens up the question as to their claims of toxic substances. Lutz's (19) claim that poisons are partially withheld when clay filters are used is certainly not true of a number of microorganisms, as shown, for example, by Young and Bennett (28).

In order to test this point further, isolations were attempted from the wilted plants of both types of filtrates. The stems of wilted plants were cut into pieces about 1.5 cm. long, the surfaces sterilized with mercuric chloride, washed in sterile water, and placed in Petri dishes containing potato-glucose agar. Out of six pieces derived from plants subjected to the filter-paper filtrate, three developed typical growth of *Fusarium vasinfectum*, while none of the pieces representing plants subjected to the filtrate which had passed through the porcelain filter showed the presence of any type of microorganism. It is not intended to suggest that cotton or filter-paper filtrates which possess poisonous properties owe their action primarily to the presence of the pathogene, but it is suggested that unless the pathogene is completely removed there is no good evidence for the toxicity theory.

EXPERIMENTS 4A AND 4B

The formula used for the Uschinsky's solution is that given in Giltner's "Microbiology," (10) under the heading "Uschinsky's asparagin medium." (There are a number of solutions going under the name of Uschinsky's solution, but as far as the writer can judge, their main point of similarity is in the name.) The test was designed to obtain further information on the difference to be expected in the toxic properties of a filtrate representing different types of media. As in nutrient-broth filtrate, there appear to be no toxic properties developed in the Uschinsky solution. It should be noted, however, that although cultures were 25 days old, growth was not as abundant as in Richards' solution. The marked sweetish odor present in the latter, as well as the amber-brown color, were entirely absent in the asparagine medium. It is also to be noted that while Richards' solution develops a hydrogen-ion concentration of around P_H 6.6, the Uschinsky solution develops marked alkalinity, P_H 8.4, in the same time interval. Probably this is due to the use of organic nitrogen in the latter medium. The difference in hydrogen-ion concentration is shown below.

Type of solution	H-ion concentration
Uninoculated Richards'	P_H 4. 2
Richards' with 2 weeks' growth of <i>Fusarium vasinfectum</i>	P_H 6. 6
Uninoculated nutrient broth	P_H 6. 8
Broth with 2 weeks' growth of <i>F. vasinfectum</i>	P_H 7. 4
Uninoculated Uschinsky's solution	P_H 8. 8
Uschinsky's solution with 25 days' growth of <i>F. vasinfectum</i> ..	P_H 8. 4

EXPERIMENTS 5A AND 5B

As a marked difference had been noted in the behavior of different strains of the cotton-wilt fungus, it seemed worth while to test the effect of a different strain from that previously described and listed as 1924 Terrace. The strain here used behaved quite differently. Wilting occurred within a few minutes, but the very bright sunlight,

as well as the high temperature present in the greenhouse at the time, may have had something to do with this, and the control (plants placed in tap water) was not considered adequate. Unfortunately, there was no uninoculated solution available at the time. Under the conditions cited above, it is quite possible that rapid evaporation may have so concentrated the filtrate as to cause wilting irrespective of any toxic ingredients. The writer suspects that the same thing occurred in a number of instances in which the investigator claims wilting in a very short period. The filtrate here discussed represented a 15-day-old culture with a marked sweetish odor, suggesting somewhat the odor of lactic acid to the writer but not to others. It had a hydrogen-ion concentration similar to the uninoculated solution, P_H 4.4, in marked contrast to the P_H 6.6 developed in the 2-weeks-old culture of the 1924 Terrace strain. Tests with ether-ferric chloride as described in Hawk's "Physiological Chemistry" (13) for lactic acid, developed a slightly greenish tinge, indicating the presence of lactic acid; however, the markedly brownish color of the medium prevented an absolute diagnosis. In any case, the quantity of lactic acid present, if any, was probably so small as to be of no particular significance.

EXPERIMENTS 6A TO 6B

The object of this group of experiments was to determine the power of the filtrate to induce wilting when it is diluted. Obviously a solution upon which a fungus has been growing for three weeks has lost part of its nutrients as well as considerable water. Also, as Brandes (4) has pointed out, there is the possibility of a greater osmotic pressure being developed in a Richards' solution with sucrose as a source of carbon because of the conversion of the disaccharide to two monosaccharides. Plants placed in the filtrate diluted with an equal quantity of distilled water showed a slight wilting in the same time interval as those placed in the undiluted filtrate, clearly indicating that it is not the possible increase in osmotic pressure that is responsible for the toxic action. On the other hand, the filtrate, diluted with twice its volume of water, possessed no appreciable toxic action.

EXPERIMENTS 7A TO 7D

It seemed worth while to determine the toxic action of a filtrate which had for its supply of carbon a monosaccharide. For this purpose chemically pure dextrose (anhydrous dextrose obtained from a commercial company) was substituted for the sucrose in the Richards' solution at the same concentration, 5 gm. in each 100 c. c. of solution. The fungus seemed to grow as well on this medium as on the sucrose medium, although no effort was made to get weights of dry fungal mats. But for some reason the uninoculated glucose medium induced a wilting, especially of the cotyledons. However, as the time of observation was increased, a difference could be clearly seen in the amount of wilting. At the end of 66 hours all the plants kept in the concentrated filtrate showed a marked wilting of the true leaves, as well as of the cotyledons; those kept in the concentrated and uninoculated Richards' solution showed only a wilting of the cotyledons; while the diluted filtrate as well as the diluted uninoculated solution showed a comparable difference. The wilting

was not as great in the diluted filtrate as in the concentrated filtrate. It appears that the filtrate possessed some toxic properties not present in the original medium, but the disadvantage of using dextrose instead of sucrose in Richards' solution appears obvious from this experiment. The hydrogen-ion concentrations of the solutions here mentioned were as follows (1924 Acala strain, 3-weeks-old cultures):

Type of solution	H-ion concentration
Uninoculated Richards' solution with dextrose-----	P_H 4. 0
Uninoculated Richards' solution with sucrose-----	P_H 4. 4
Three-weeks-old culture on dextrose-Richards' solution---	P_H 6. 8
Three-weeks-old culture on sucrose-Richards' solution----	P_H 5. 0

A comparison of these data with the hydrogen-ion data previously given indicates that at the end of the same time interval there is a noticeable difference between the hydrogen-ion concentration in this strain of *Fusarium vasinfectum* and the other strain.

EXPERIMENTS 8A TO 8F

Livingston (18) has shown that sunlight undoubtedly influences the rate of transpiration, and it appeared desirable to test its action on the rate of wilting. This series of experiments apparently brings out quite clearly the fact that very bright sunlight affects the rate of wilting, for in one filtrate exposed to strong sunlight plants wilted in approximately one-fourth the time required by plants in a similar filtrate in diffused light. The explanation would appear to be as follows: As transpiration increases, there is a greater intake of water and probably of the dissolved substances, and this increase would account for the more rapid wilting. That it is not due merely to a concentration of the filtrate outside of the plant appears to be evident from the fact that control plants in the uninoculated solution do not wilt under the same conditions in the same length of time.

EXPERIMENTS 9A TO 9F

This series of experiments was designed to help in determining the nature of the toxic substance. Was it volatile, and was it thermolabile? About 300 c. c. of the filtrate which had passed through a Berkefeld filter was subjected to distillation, for the first 30 minutes at about 100° C., and later at 110° to 120°. The first distillate was collected in about 100 c. c. of cold distilled water, the point of the condenser resting below the water level so as to prevent the escape of any gas. The odor of the first distillate was very sharp, pungent, and somewhat sweetish. Tests for amines with gold chloride and with chloroplatinic acid were negative. The odor of the second distillate was not as distinct as the first. The residue, consisting of 75 c. c., contrasted with the original 300 c. c., had a dark-brown color and a strong caramellike odor. Plants placed in the first distillate wilted within 24 hours, while control plants placed in uninoculated Richards' solution remained turgid. In contrast to this, the plants placed in the second distillate showed no wilting in the same period. Evidently the volatile substance possessing toxic properties passed off quickly and completely. However, neither of the distillates possessed the extreme toxicity of the residue. When this was diluted with five parts of water, the plants subjected to it showed signs of flagging within several hours, in spite of the fact that the dilution was

more than sufficient to make up the original volume. Even the residue diluted with 20 times its volume of water showed toxic qualities almost equal in severity to those of the lesser dilution. Dilutions making a 2 per cent and 1 per cent solution of residue possessed but slight toxic properties. Compared to these, the first distillate appeared more toxic. From this experiment one may conclude as follows: There are two toxic substances present in the filtrate, one volatile and possessing slight toxic properties and the other non-volatile, thermostable, and increasing in toxicity upon heating. Tests for amines were undertaken because a number of these are known to be highly toxic, some of them resulting from the action of microorganisms. The interesting work of Krueger and Alsberg (16) in detecting mono-, di-, and triamines in cultures of *Pseudomonas solanacearum*, a wilt producer, also indicated possibilities for their detection in the media sustaining the cotton-wilt fungus. While the tests previously cited are commonly used for detecting amines, including the closed ring ones, the alkaloids, the writer does not feel that the negative results obtained are conclusive. The detection of various amines in the filtrate of *P. solanacearum* does not necessarily mean that these substances have anything to do with wilting, and Krueger and Alsberg make no such claim. The fact is that amines are not known to have any definite physiological function in plants, including the microorganisms, and are found constantly in only a small proportion of them.

EXPERIMENTS 10A TO 10C

It seemed worth while to test a third strain of the cotton-wilt fungus since the two others had shown such divergence in color developed in the media and in the hydrogen-ion concentration. A 3-weeks-old culture tested P_H 6.2, while the uninoculated control of the same age tested P_H 4.0. In all respects the filtrate closely resembled the 1924 Terrace strain. Krueger in correspondence had suggested testing for nitrites in the filtrate. Such tests are common in bacteriological work, but rather rare in work with fungi. Using the sulphanilic acid-alpha naphthalamine method, a very positive test for nitrites was obtained in the filtrate, while the uninoculated Richards' solution gave entirely negative results with no indication of a pink color. The test was repeated several times and with the same result. There is no doubt that in a medium containing nitrate the fungus is capable of reducing this to nitrites. Furthermore, it was interesting to see that a 3-weeks-old culture of the same strain, growing upon Uschinsky's solution, gave only negative results when tested for nitrites. It will be recalled that with the latter medium the filtrate is not toxic to cotton and that the medium itself contains no nitrates.

Of the 9 plants placed in each of the different solutions represented in experiments 10a, 10b, and 10c, 5 were about 10 inches high and the 4 remaining were seedlings. Was there any difference in toxicity to be noticed in older plants compared to seedlings? The results show that while the seedlings wilted completely within five hours in the unboiled filtrate and were quite flaccid in the boiled filtrate, the larger plants, with the exception of one in each, showed no such ill effects. The larger plants were not as thrifty as the seedlings,

and this fact may have influenced the results. Eventually the larger plants also wilted, but whether this was due to the filtrate or to some secondary organism introduced into the filtrate was not determined. It should be noted that bacteria of various sorts thrive very well on the filtrate when they have considerable difficulty in growing in the uninoculated solution. The reason doubtless is to be found in the hydrogen-ion concentrations, considerable acidity, P_H 4.0, of the uninoculated medium acting as the deterrent. The control plants in the uninoculated medium remained turgid and healthy looking for about 48 hours. Irrespective of poisons, the writer finds that it is more difficult to keep plants thrifty in water solutions during the warm parts of the year than during the cooler parts. As has already been shown, the boiled filtrate also possessed toxic properties. The filtrate in a cotton-stoppered Erlenmeyer flask was heated over a water bath for about 20 minutes at $100^{\circ}C$. However, the amount of flaccidity and wilting in the five-hour period was not as great in the boiled filtrate as in the unboiled, indicating that the heating may have driven off some of the volatile toxic substance. Boiling, as reported elsewhere, does not destroy the toxic principles.

EXPERIMENTS 11A TO 11D

It has already been recorded that filtrates of the tomato-wilt fungus, *Fusarium lycopersici*, exercise but slight toxic effects upon cotton plants. Would the filtrate from a fungus that is more closely related to *F. vasinfectum* be more toxic? The cowpea-wilt fungus, *F. tracheiphilum*, is so closely related to the cotton-wilt fungus that for a long time they were considered as one species. Indeed, the main difference known at present is the difference in susceptible hosts. This experiment indicates that the filtrates of the two possess about the same toxicity to cotton. This might be interpreted to mean that studies on filtrates are not conclusive for toxicity study, inasmuch as they are not specific, as Lutz (19) and Bisby (3) have pointed out. There is, however, another possible explanation. When one severs the root-system of a plant, or uses an excised leaf as Bisby did, any selective action is of course largely destroyed for that plant. But irrespective of this, we are dealing here with parasites that push their way into and grow rather profusely within a susceptible plant. In spite of the fact that the group of *Fusaria* as a whole are remarkable in their range and frequency, easily living on almost all kinds of dead matter, they show marked restrictions in their capacity to parasitize, often limiting themselves to but one species or only to certain varieties of that species, and then only attacking under certain restricted conditions. In other words, the possibility of filtrates of various and diverse fungi causing wilting of a given plant does not necessarily overthrow the experimental data obtained in filtrate studies of the parasite specific to that plant. One must first show that a certain fungus is capable of penetrating and living within a given plant before toxicity studies of its filtrates are rejected or accepted.

EXPERIMENTS 12A TO 12F

These experiments were undertaken to find out more about the substances in the filtrate of Richards' solution which possessed toxic properties. As the filtrate was to be heated, the fungus was filtered

off by means of fine filter paper. In spite of the fact that the solution was markedly less acid, judged by the hydrogen-ion concentration, than the uninoculated solution, yet there was the possibility of the formation of organic acid having a low dissociation constant, together with other metabolic products possessing alkaline properties. At the suggestion of A. P. Krueger, strictly chemically pure calcium carbonate was added to the filtrate, together with a few drops of toluol. After standing 48 hours, the carbonate was filtered off and the filtrate was concentrated by heating and distilling in vacuo. Krueger had suggested that such a concentrated filtrate might in time develop crystals representing calcium salts of any organic acids which might be present. At the same time, it seemed desirable to gather the distillates in order to check the former distillation experiment. The solution was boiled at about 90° C., and the distillate collected during the boiling. It appeared as a colorless liquid with an odor similar to that previously described for distillates. It was slightly alkaline, testing P_H 7.5. As indicated in the table, it possessed toxic properties, although the wilting of the plants was not as severe or as rapid as in the residue. Another portion of the residue which was left standing for several days in an open dish, the sides of which were rubbed occasionally with a glass rod, failed to show any crystals. As the distillate gave an alkaline reaction, it seemed worth while to test for organic bases. But tests for amines, previously cited, were negative. As Lathrop (17) had reported the finding of aldehyde in a filtrate of *Fusarium cubense* growing upon an Uchinsky solution, tests for aldehydes were made, including the resorcinol test for aliphatic aldehydes, the acenaphthene test for cyclic aldehydes, and the silver-mirror test. All were negative.

The chemical investigation of staling in *Fusarium* sp. conducted by Pratt (24) is of considerable interest. When fungi were grown upon Richards' solution, she found that they produced small quantities of alcohol, salts, fatty acids, ammonia, and possibly traces of aldehydes. Staling was not considered to be due to an enzyme, but rather to the simple organic acids which are toxic at low concentrations. In spite of the failure of the writer to obtain calcium salts of organic acids, the subject is worthy of further investigation. Unfortunately, the rush of other work has for the present made impossible any further efforts in this direction.

The positive tests for nitrites in filtrates of 2 or 3 weeks old cultures of *Fusarium vasinfectum* growing upon Richards' solution made it desirable to find out more about this chemical. It is known that nitrites are quite toxic to plants at fairly low concentrations. Was the concentration in the filtrates great enough to be poisonous? Using the official method for the quantitative determination of nitrites, it was found that with three different strains tested at different times the amount of nitrite-nitrogen varied from 0.0125 mgm. to 0.04 mgm. per cubic centimeter. A solution of chemically pure sodium nitrite was made up so as to contain 0.04 mgm. of nitrogen per cubic centimeter. Six cotton seedlings with roots cut under water were placed in this solution, and within 22 hours a marked withering and browning of the stems was noticed, starting at the base and running up for some distance. But when the experiment was duplicated and plants were placed in the solution which had been

made up about 48 hours previously, there was no ill effect to be noted. Nitrites in solution are not very stable, and this probably accounts for the seeming discrepancy. That nitrites in dilute form can produce a wilting and killing of cotton plants was determined in the following manner: One pot of cotton seedlings growing upon a sandy loam soil received 1,000 c. c. of a sodium nitrite solution containing 0.5 mgm. of nitrite-nitrogen per cubic centimeter. The solution was applied in three applications at intervals of 24 hours. Another pot was treated in a similar manner, using potassium nitrite as the salt. A third pot, treated with water, served as a control. Within 48 hours all of the plants, about 40 in number, of the two pots treated with nitrites (see fig. 2) showed droopy cotyledons and



FIG. 2.—Effect of nitrite on cotton plants. Control pot at the right and pot to which 1,000 c. c. of sodium nitrite, 0.5 mgm. of nitrite-nitrogen in each cubic centimeter, at the left. Plants all of the same age and handled similarly. Photographed 48 hours after initial treatment. Twenty-four hours later all the plants in the nitrite pot were dead.

leaves, while those of the control pot remained normal. Within three days after the applications, almost all of the plants in the nitrite pots were dead. When one considers the instability of nitrites under soil conditions, the greater concentration applied to soil as compared to those found in the filtrates does not seem objectionable.

The experiment clearly indicates that in the presence of nitrates the fungus produces nitrites which may in part cause the pathological effects. As the fungus is a vascular parasite, it must undoubtedly at times come in contact with nitrates taken up by the roots. Furthermore, as the same fungus is a soil inhabitant, it is quite possible to conceive of nitrite poisoning readily occurring in the absence of any fungal invasion.

OTHER EVIDENCE INDICATING TOXIC ACTION

Aside from evidence of toxic action deduced from a study of filtrates, there is other evidence indicating that wilting is not due to a mere mechanical plugging of the vascular elements by the fungus. It has already been noted that Ajrekar and Bal (1) found that by far the greatest number of vessels in a wilted cotton plant are not occupied by the fungus. The writer likewise has spent considerable time in making microscopic observations of wilted cotton plants and can confirm Ajrekar's and Bal's findings. In addition to this, the writer has not infrequently been unable to culture the fungus from wilted plants which possessed discolored vascular elements, even when stem bases and pieces of taproots were utilized. That other investigators have been unable at times to culture *Fusarium vasinfectum*



FIG. 3. --Front row: Nine plants with roots removed, after standing 24 hours in uninoculated Richards' solution. Back row: Nine plants with roots removed, after standing 24 hours in a filtrate of Richards' solution in which *Fusarium vasinfectum* had been grown for 3 weeks

from wilted cotton plants is, in view of these observations, not surprising, and Dastur's (6) conclusion that wilt is not caused by that fungus was due partly to his inability to culture the fungus from some wilted plants.

CONCLUSION

A study of filtrates with Richards' solution as the medium for the growth of *Fusarium vasinfectum* shows that there are at least two toxic substances present in the filtrate. (See fig. 3.) One is a volatile compound, slightly alkaline in reaction, and the other an inorganic salt, in the form of nitrite. There may be other toxic substances, such as organic acids or alkaloids, but these have not been detected. The finding of nitrites in sufficient quantity to cause poisoning of

cotton plants opens up a field of investigation which has largely been neglected in work with wilt-producing fungi.

As the fungus is not present in sufficient quantity within the xylem to account for the wilting, and as wilting is at times obtainable in the absence of the fungus, it seems logical to conclude that the pathological phenomena here discussed are due primarily to certain poisonous chemical compounds produced by the fungus.

SUMMARY

A large number of experiments are recorded in which pure cultures of different strains of the cotton-wilt fungus were grown on various liquid media, the fungus removed from these, and toxicity studies undertaken with the filtrates.

It is shown that filtrates representing cultures growing on Richards' solution are quite toxic to cotton plants, while filtrates of cultures growing on media containing organic nitrogen, such as Uschinsky's asparagine solution or peptone-beef broth are nontoxic. May not this explain why cotton wilt is much more prevalent in light sandy soils devoid of appreciable quantities of organic nitrogen as compared to richer types of soils? Attention is called to the difference in metabolic products to be expected in media of diverse composition.

When cultures are filtered through fine filter paper the filtrate is found to contain viable spores. It has been determined that while the sterile Richards' solution is markedly acid, testing P_H 4.0 to P_H 4.4, the growth of the cotton-wilt fungus for two to three weeks renders the solution considerably less acid, the hydrogen-ion concentration ranging from P_H 6.0 to P_H 6.6. It appears, however, that this change in acidity is not the factor which renders the filtrates toxic.

When whole plants with root systems carefully handled are placed in filtrates of *Fusarium vasinfectum*, wilting ensues as with plants that are freed from roots.

That the toxic properties of filtrates of Richards' solution are not due to increases in osmotic pressure was determined by diluting the filtrates and observing its action on cotton plants.

Substituting glucose for sucrose in Richards' solution, it is found that the fungus makes as good a growth as in the sucrose medium and that the glucose medium also possesses toxic properties. It was found that uninoculated glucose Richards' solution caused a slight withering of the leaves.

In very bright sunlight, it is recorded that filtrates cause wilting much more rapidly than in diffused light.

By subjecting filtrates to distillation in vacuo as well as to ordinary boiling and testing, the distillates as well as the residues, were found to possess toxic properties, the residue being considerably more toxic. The distillates possess a pungent odor and give an alkaline reaction. Tests for amines and alkaloids in the distillates were negative.

The filtrates of 2 or 3 weeks old cultures on Richards' solution gave positive tests for nitrites. These were found in quantities ranging from 0.0125 mgm. to 0.04 mgm. of nitrogen for each cubic centimeter of solution. Using chemically pure sodium nitrite solution

comparable to the concentration found in the filtrates, it was determined that this is markedly toxic to cotton plants.

Comparing the action of filtrates of three different species of *Fusarium* on cotton plants, it was found that a filtrate of *F. tracheiphilum*, the cowpea-wilt fungus, is comparable in toxicity to filtrates of *F. vasinfectum*, while a filtrate of *F. lycopersici*, the tomato-wilt fungus, is considerably less toxic. Attention is called to the close relationship existing between the two first-mentioned species.

Attempts to obtain calcium salts of any organic acid present in the filtrate of the cotton-wilt fungus resulted in failure.

Microscopic observations of the vascular elements of wilted cotton plants clearly indicate that wilting is not due to a mechanical plugging up of the vessels by the fungus.

It is recorded that wilted cotton plants are occasionally found in fields badly infested with the wilt-inducing fungus from which the fungus is apparently absent. It is suggested that the wilting and interior discoloration of the xylem in these cases is due to the formation of toxic substances by the fungus in the soil.

The conclusion is drawn that filtrates of *Fusarium vasinfectum* growing on Richards' solution possess at least two substances poisonous to cotton. One is a volatile compound with an alkaline reaction and the other is an inorganic salt in the form of nitrite.

The wilting of cotton infected with *Fusarium vasinfectum* is considered to be due to poisonous chemical substances formed by the fungus.

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CORRELATED INHERITANCE IN WHEAT¹

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INTRODUCTION

Since the rediscovery of Mendel's law in 1900, there have been many studies of inheritance in wheat. Of these rather numerous investigations, however, only a few have comprised correlated studies of various characters on the same plant.

Inheritance of awns and of spike density have received some attention, but the studies have hardly more than indicated the problem. Both have been thought by some to be rather simple in their inheritance, and in some crosses such is the case without doubt, but lately there has come a recognition of considerable complexity.

As used in this discussion, the term "density" refers to compactness of the spike. Compact spikes have short rachis internodes, and the spikelets are close together. Such a spike is said to be "dense," whereas one with long rachis internodes, with the spikelets far apart, is said to be "lax."

REVIEW OF LITERATURE

The literature available on the inheritance of awns and spike density in wheat is surprisingly meager. Only four applicable papers on awns and a somewhat greater number on spike density have been found.

AWNS

It has been generally accepted that the awnless condition is dominant over the awned condition in wheat and that the F_1 plants are intermediate in this respect. Several workers report a simple one-factor difference and therefore obtain a 3:1 ratio or a 1:2:1 ratio.

Howard and Howard (7)³ in India found that some wheats behaved in a considerably more complex manner. A fully bearded wheat was crossed with one described by them as being really awnless, a fact which is emphasized as important, inasmuch as the so-called awnless varieties really have short tip awns. In the F_2 , five awn classes were obtained: (1) Entirely awnless, (2) short tips, (3) long tips, (4) nearly fully bearded, and (5) fully bearded. When the awned and partially awned plants were grouped together, there was a ratio of 15 awned to 1 entirely awnless. These results were explained on a two-factor basis. Four classes bred true, and when the short-

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² The writer wishes to express his appreciation for the helpful suggestions of H. K. Hayes concerning the plan of the experiment and the methods of studying the data. Thanks are due to E. C. Stakman and M. N. Levine for cultures of physiologic forms of black stem rust of wheat and for growing two hybrids in the international uniform rust nursery; to O. S. Aamodt for assisting in various ways with the rust investigations; and to D. C. Tingey for growing the various generations of hybrids and for assistance in recording observations and in making calculations.

³ Reference is made by number (italic) to "Literature cited," p. 1191.

tipped plants and the long-tipped plants were crossed, F_2 segregation showed some fully bearded and some awnless plants as well as the intermediate forms.

Clark (4) likewise found a somewhat complex condition in the inheritance of awns of a cross between Hard Federation and Kota. He made five classes of awn types in F_2 and arrived at the conclusion that segregation was too complex to analyze by the methods which he used. He reached the conclusion also that awns followed to a certain extent the maternal parent. This was somewhat irregular, but he felt that his results justified his conclusion.

Recently Nilsson-Leissner (12) and Meyer (9) have shown in spelt \times vulgare crosses that awns are linked with the spelt and with the speltoid forms.

Nilsson-Ehle (11) obtained by mutation true-breeding forms of awnless, of half-awned, and of awned wheats. Awnless forms were partly dominant to half-awned and to awned, and half-awned forms to awned. He explained his results on the basis of multiple allelomorphs for half-awned and fully awned plants arising by complex mutation from the awnless plants.

SPIKE DENSITY

In crosses between species of wheat, rather dense spikes have been obtained in unusual ratios, but usually when vulgare wheat has been studied in a cross with other vulgare wheats or with true *Triticum compactum*, rather simple segregations have been found. There are, however, a few complex cases.

Spillman (14), Gaines (5), Biffen (1), and others report simple segregations into 3:1 or 1:2:1 ratios.

Hayes and Harlan (6) report that barley internode length is a stable character and segregates in a distinctly reliable manner, a variety of homozygous lines being established in F_3 . From crosses between two sorts varying in spike density they obtained true-breeding lines, similar to both parents, in one case without any homozygous intermediate forms and in another case with true-breeding intermediates. Their genetic analysis seems to indicate one main-factor difference in one cross, two factor or three factor differences in others, and in some cases in addition to the main-factor differences some minor modifying factors.

Boshnakian (2) found spike density correlated with length of straw. He concluded that compactness in spikes was correlated with a tendency to shorten the plant throughout. Squareheadedness was defined as a condition wherein the number of rachis internodes in the upper third of the rachis bore a ratio of not less than 1.33 to the number in the middle third. Squareheadedness was somewhat affected by environment but was found to be inherited.

Parker (13) crossed a *compactum* wheat with a squareheaded vulgare form of intermediate density and obtained forms decidedly more lax than either parent. There was a series of forms of increasing density until the density of the compact parent was reached. Parker thought his paper "clearly pointed out the fact that the problem of the inheritance of the character of laxness and denseness in the ears of wheat is a much more complicated one than was previously imagined." The importance of determining density by measuring internode length instead of making "eye classification" is emphasized.

Nilsson-Ehle (10) obtained dense, mid-dense, and lax forms from a cross between compact and mid-dense sorts. The mid-dense parent (squarehead) was recovered in about 1 of every 64 F_2 plants. He explained his results by designating the compact parent as CC L_1L_1 L_2L_2 and the mid-dense parent as cc l_1l_1 l_2l_2 . L_1 and L_2 were lengthening factors and C was a factor which produced short rachis internodes and also inhibited the expression of L_1 and L_2 .

More recently Nilsson-Ehle (11) has reported a mutation of speltoid types in a progeny of a cross of spelt \times vulgare. One of his speltoid types again mutated to produce a subcompactum (11). Nilsson-Leissner (12) also reports similar findings as well as several chimeras in spike density and in branched spikes (ramification).

Meyer (9), who measured the length of rachis internodes in several crosses, found in one of them a 1:14:1 ratio of pure compact, intermediate and heterozygous combined, and pure lax. White Frankenstein (internode length of 5.3 mm.) was crossed with Eckendorfer (internode length, 3.75 mm.). The F_2 plants segregated in forms varying in spike density from 3.74 mm. to 4.94 mm., with the greater number of plants showing intermediate density.

EXPERIMENTAL DATA

The research work herein reported falls naturally into two parts, as follows:

(1) General plant-breeding work on a cross between Dicklow and Sevier varieties of wheat. In this cross detailed genetic data were not obtained, but some yield data were obtained and some strains which were thought to be resistant to *Puccinia graminis tritici* E. & H.

(2) Detailed study of single and correlated characters in a cross between Sevier and Federation varieties of wheat.

DESCRIPTION OF PARENTS

The parents used were Dicklow, a spring wheat commonly grown under irrigation; Federation, a new variety introduced rather recently from Australia; and Sevier, a variety discovered about as recently in Sevier County, Utah.

DICKLOW

Dicklow wheat possesses the spring habit of growth and is medium late in maturing. The stems and leaves display a rather grayish-blue color just before maturity owing to a distinct glaucousness. The stems are rather strong and coarse, and the leaves are broad. The spikes are awnless, though short beards or beaks are rather common at the apex. The glumes are glabrous and white. The kernels are soft, white, and from short to mid-long. The spikes vary from an erect to a nodding position. The shape of the spike most common to the variety is mid-dense and clavate (clubbed at the apex). This latter character varies, depending on environment.

FEDERATION

The variety Federation has great commercial importance in Idaho on irrigated land and is now being brought into Utah, where it is replacing the predominant variety Dicklow on land where lodging is bad. Federation has better standing ability and was therefore chosen as a parent to cross with Sevier, whose standing ability is markedly weak. Federation has a distinct spring habit, matures somewhat earlier than Dicklow, is about 15 to 20 cm. shorter of culm than Sevier and about 6 to 8 cm. shorter than Dicklow.

Its spikes are awnless except for very short tip awns and an occasional apical awn, and the variety is classified by Clark, Martin, and Ball (3) as awnless. The spike is somewhat oblong, a trifle more dense than ordinary lax wheats such as Marquis, and is borne erect. The glumes are dark bronze to brown and the kernels are white, soft, and short.

SEVIER

The variety Sevier has commercial importance in the Sevier River valley of Utah where there is some black stem rust occasionally. The straw is extremely weak and the grain lodges badly, although unless lodging is extremely severe, there is little loss, for Sevier seems to have a certain amount of resistance to the physiologic forms of *Puccinia graminis tritici* that occur in this region. It is extremely high yielding under favorable conditions, is somewhat drought resistant, and is also thought to be slightly resistant to alkali, though this has not been proved. The spike is awned and is somewhat laterally compressed, and though it is somewhat more dense than the spike of Federation, Sevier can not be classed as a club wheat. The glumes are bronze but not so dark as those of Federation. The kernels are white and in some pure lines are almost as hard as those of durum wheats, whereas in others the kernels are soft. In the pure lines used in the hybrids studied the kernels are hard but not so hard as in some of the other lines. The grain is held firmly in the chaff and no amount of weathering seems to cause shattering, a fact which makes it a desirable parent to use both with Federation and Dicklow, as these two varieties both lose considerable grain if left standing in the field any length of time after maturity.

DICKLOW×SEVIER CROSS

In 1919, in an effort to obtain improved strains of wheat, a large number of variety crosses were made between Sevier and Dicklow, the foremost spring wheat grown under irrigation in this region. Both varieties were composite; that is, each contained several or many pure lines, some of which were distinctly visible. In this first cross no record was made as to which plants were used as parents. F_3 and F_4 homozygous lines were obtained, some of which were more dense and others more lax than either parent. Pure-line selections were made in both varieties, and numbers were assigned to each. In 1921 and subsequently, yield data were obtained on these pure lines and on hybrid strains as soon as they became available.

In 1924 nursery tests were made under irrigation and in 1925, under dry farming. Table 1 gives the yield data for the irrigation test and Table 2 those for the dry-farm test.

In both tables it is apparent that high yields and good straw strength were obtained in certain of the hybrids. In both cases, also, some of the best-yielding lines have weak straw. In the dry-farm tests, Sevier yields were very high, but this wheat is excessively weak-strawed. There is considerable economic promise in the hybrids both for irrigated farming and for dry-farming. For example, IV C 15, III C 8, and F 68 were thought to be promising and are now being tested in plats. In the tests with irrigated spring wheat, III C 18, F 22, F 68, and V C 8 have been used in plot tests. G 149 has proved very rust resistant.

TABLE 1.—Yields of superior strains of *Dicklow*×*Sevier* hybrid wheats, odds that they will be better yielders than *Dicklow*, shape of spike, awn condition, and straw strength (perfect erectness=100 per cent)

[Irrigated spring wheat, 1924; average of four 3-row blocks, at Logan, Utah]

Spike shape	Pedigree	Awns	Acre yield	Per cent erectness	Odds
			<i>Bushels</i>		
Compact club	III C 18	a A	70.1±3.35	83	1500:1
Do	G 38	b a	68.9±3.29	76	
Medium club	G 205	a	68.5±3.27	62	
Faintly compact	F 68	A	68.5±3.27	83	
Compact club	P 72	A	67.9±3.25	71	
Do	F 22	A	67.1±3.21	92	
Medium club	G 148	c A	67.0±3.20	75	400:1
Long loose	G 218	A	67.0±3.20	70	
Compact club	IV C 2	A	66.8±3.19	85	
Medium club	G 97	a	66.7±3.19	78	
Faintly compact	C 18	A	65.9±3.15	57	
Medium club	C 10	A	65.7±3.14	75	
Compact club	V C 8	A	65.7±3.14	80	
Do	D 10		65.3±3.12	74	
Long loose	G 175	a	65.0±3.11	82	
Do	G 209	a	65.0±3.11	56	145:1
Medium club	F 32	A	64.7±3.09	80	142:1
Compact club	F 24	A	64.6±3.09	89	
Long loose	G 149	A	64.5±3.08	61	
Do	A 4	A	64.5±3.08	78	
Do	A 4	A	64.5±3.08	78	
Do	G 112	a	64.3±3.07	47	
Do	P 42		64.0±3.06	77	
Do	G 27	A	63.8±3.04	80	
Medium club	D 9	Aa	63.5±3.04	76	
Long loose	G 49	A	63.1±3.02	73	
Do	C 10		63.1±3.02	82	100:1
Do	G 31	A	63.0±3.01	77	
Do	C 22	a	63.0±3.01	65	
Compact club	P 14	Aa	62.3±2.97	80	
Long loose	G 212	A	62.0±2.96	48	
Do	G 121	a	61.8±2.95	43	
Compact club	G 180	A	61.7±2.95	75	
Do	G 84	A	61.7±2.95	80	
Medium club	G 11	Aa	61.6±2.94	80	
Do	C 14		61.5±2.94	78	
Do	C 9		61.2±2.93	82	
Faintly compact	D 5	A	61.2±2.93	61	
Compact club	G 122	A	61.0±2.92	80	
Do	V C 1	A	61.0±2.92	79	30:1
Do	G 126	A	61.0±2.92	75	
Do	Dicklow		49.2±2.35	79.6	(Av. 50)

a A=awnis

b a=awnless.

c Aa=heterozygous for awns.

TABLE 2.—Acre yields and percentage erectness of pure lines of Sevier, of hybrid strains of Dicklow×Sevier, and of the commonly grown dry-farm varieties

[Dry-farm winter wheat; average of three 3-row blocks grown in 1925 at Logan, Utah]

Strain	Acre yield	Per cent erectness	Strain	Acre yield	Per cent erectness
	<i>Bushels</i>			<i>Bushels</i>	
Sevier 86.....	60.1±3.08	40	Sevier 34.....	48.6±2.49	40
V C 8.....	58.7±3.01	58	F 22.....	47.8±2.45	83
IV C 15.....	57.7±2.96	85	Kofod.....	47.6±2.44	7
V C 2.....	57.6±2.92	33	S 33.....	47.6±2.44	88
Sevier A.....	56.9±2.92	25	P 14.....	47.4±2.43	88
P 72.....	56.8±2.91	62	Sevier 59.....	47.3±2.43	23
Sevier 161.....	55.7±2.86	23	Sevier 56.....	47.0±2.41	38
III C 8.....	55.4±2.84	83	S 26.....	46.7±2.40	85
S 27.....	55.3±2.84	75	G 40.....	46.4±2.38	45
G 33.....	54.7±2.81	77	R 5.....	45.9±2.35	20
Sevier 125.....	54.5±2.80	20	S 39.....	45.8±2.35	92
F 68.....	54.4±2.79	50	Sevier 31.....	45.8±2.35	12
Sevier 40.....	53.9±2.76	25	Turkey.....	45.7±2.34	38
III C 18.....	53.4±2.74	63	P 6.....	45.3±2.32	85
Kanred.....	53.4±2.74	20	G 122.....	44.2±2.27	83
Sevier 47.....	52.9±2.71	38	Odessa.....	43.4±2.23	25
Sevier 128.....	52.5±2.69	33	G 175.....	42.7±2.19	70
IV C 9.....	51.4±2.65	92	R 128.....	42.3±2.17	80
Sevier 83.....	51.4±2.64	8	S 21.....	41.0±2.15	78
IV C 2.....	51.3±2.63	87	Silver Coin.....	40.6±2.08	87
G 84.....	51.3±2.63	60	R 152.....	40.1±2.06	83
F 32.....	51.2±2.63	58	G 148.....	38.9±2.00	83
P 144.....	51.1±2.62	90	Gold Coin.....	38.1±1.96	92
P 42.....	51.1±2.62	75	F 42.....	37.3±1.91	68
G 149.....	48.8±2.50	50	G 97.....	35.6±1.83	88
F 24.....	48.8±2.50	77	Jones Winter File.....	35.3±1.81	78

RUST TESTS

In 1924, 55 pure lines from the cross Dicklow×Sevier were grown in the rust nursery at University Farm, St. Paul, Minn. Of these, two were found to be highly resistant. Three or four were segregating for resistance, and all the others were highly susceptible. The two resistant strains were G 40 and G 149.

During the spring of 1925 seedlings of those two strains were subjected to greenhouse rust tests in which all the physiologic forms of black stem rust of wheat then available in the pathology greenhouse at University Farm were used. Altogether 19 forms of rust were employed. Under each variety in Table 3, where the data are tabulated, the types of seedling infection are listed. Type 4 is a large uredinial pustule with little or no chlorosis. It indicates complete susceptibility. Type 3 is a smaller pustule with some chlorosis. Type 2 is a small pustule inclosed in a considerable area of hypersensitive tissue. Type 1 is an extremely small pustule with a distinct margin of necrotic tissue. The most resistant type is not a pustule at all, but only sharp flecks, indicating that the rust has infected the leaf but has been overcome and thrown off. Such infection is designated "zero flecks" (0 f).

TABLE 3.—Relative resistance of two Dicklow \times Sevier hybrids, as compared with that of their parents and of Marquis, to various physiologic forms of *Puccinia graminis tritici*

[Tests at University Farm, St. Paul, Minn., 1925]

Physio- logic form	Date observed	Marquis seed- lings showing infection of type--				Dicklow seed- lings showing infection of type--				Sevier seed- lings showing infection of type--				G40 seedlings showing infection of type--				G149 seedlings showing infection of type--				
		0f	1	2	3	4	0f	1	2	3	4	0f	1	2	3	4	0f	1	2	3	4	
1	May 15				12					11				12			15			14		
	June 20				10															8		
3	May 16									10												
	June 18									11										11		
	do.																			10	1	
9	May 12				8					11				12			4	5		1	2	3
	June 20									10										13		
	May 19									12									2	7		
10	June 19									11										10		
	do.									12										10		
11	May 14									14			4	8			2	13	0	4		
	June 17									14										15	1	
15	May 17									9				7	3		4	5	2	10		
	June 20									13										16		
Pusa 17	May 23									14				6	7		4	4		5	6	
Minn.	June 1				15					10				14			13			16	Photo	
Pusa	June 18									11									10		2	
	June 11				11					110				8	5		2	10		9	3	
18	June 19									12										13		
	do.									13										14	1	
19	June 10									11				10	3		2	13		2	10	
	June 17									13										11		
21	May 24				5	7				11				8	4			9	3		11	
	June 17									13										12		
24	June 22									10										10		
27	May 30								1	2	7			11	2		2	5	5		2	
	June 18									13										5	8	
29	May 25									14				2	13		1	8	3		11	
	June 17				13					12										11	3	
30	May 20									15				14						13	1	
	June 22				13					13									2	13	1	
32	June 10									11				9			9			13	7	
	do.																			11		
	June 22									8							10			11		
36	May 8									14			6	5			6	3	1	8	2	
	June 19									12										12	5	
	do.																			14	1	
38	May 16				12					13				12						16		
	June 18									12										11		
39	May 18				10					14				13			4	11		12	2	
	June 18									10				11						10	3	
40	May 23									14							5	6		13	9	
	June 19									9										13		

Dicklow was found to be completely susceptible to all 19 physiologic forms of *Puccinia graminis tritici*, whereas the Sevier parent showed some resistance. Pure line G 40 showed about the same resistance as did Sevier. Pure line G 149, however, was on the whole considerably more resistant than either parent (see fig. 1). It is worthy of notice that G 149 was resistant to every form with which it was inoculated, which included all of the forms that have been known to cause epidemics in the United States. It is notably resistant to rust forms 9, 17, 32, and 36, the last one of which severely damages Kanred. Because it arose in a varietal cross the exact percentage of G 149 is not known.

Leaf-rust observations were also taken in 1925 at University Farm. About 75 per cent of the Marquis wheat was infected as compared with 80 per cent of the G 40 and 22 per cent of the G 149. Tests by Melchers of 13 strains of the same cross in Kansas in 1924 showed 10 completely susceptible, 1 slightly resistant, 1 moderately resistant, and 1 resistant, i. e., "many sharp flecks, few minute uredinia."

Just occasionally, it seems, there occurs in the progeny of this cross a plant resistant to stem rust and to leaf rust.

In 1925 another set of pure lines from the Dicklow \times Sevier cross was grown in the rust nursery at St. Paul. G 40 and G 149 were included and were again found to be highly resistant. One additional pure line, G 84, was found to be resistant, fully as resistant so far as this one test is concerned as G 149. Many other pure lines were segregating for resistance to rust. Resistant plants from the segregating row of G 212 grown in 1924 were sown separately in 1925.

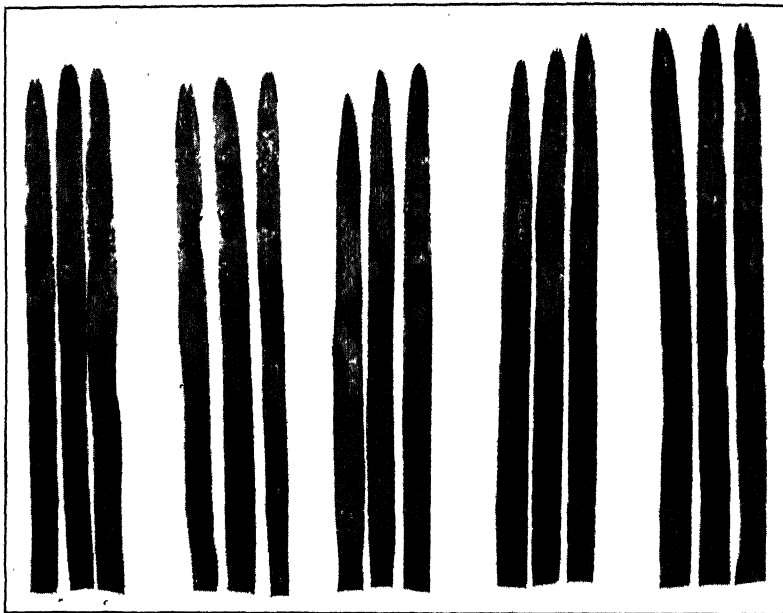


FIG. 1.—Reaction of Marquis, Dicklow, hybrid G 149, hybrid (140), and Sevier wheats to physiologic form 17 of *Puccinia graminis tritici* in the greenhouse. Marquis (extreme left) was used as a control and gave a 4 reaction. Dicklow, one of the parents (second from left), gave a strong 4 reaction (fully susceptible). Sevier, the other parent (extreme right) gave a 3 reaction (somewhat resistant). One of the hybrids between Dicklow \times Sevier, G 40 (second from right), gave a 1 reaction. The other hybrid, G 149, gave 1 and “zero-fleck” reactions (highly resistant). G 149 was moderately to highly resistant to all the 19 physiologic forms available for test.

All of these were infected to about the same extent as G 40, G 84, and G 149. The artificial epidemic in 1925 was produced by spraying with spores of physiologic forms 1, 3, 9, 15 (?), 17, 18, 19, 21, 29, 30, 36, 38, and 39.

During the summer of 1925 both pure lines G 40 and G 149 were grown in the international uniform rust nurseries of the United States and Canada. A summary³ of the tests is given in Table 4. Ceres, Kota \times Marquis, Marquillo, Kota, and Progress showed marked resistance as compared with Marquis. The percentages of infection in Kota, Kota \times Marquis, and Ceres were, respectively, 6.59, 4.92, and 8.62, as compared with 26.60 in Marquis and 30.89 in Preston. In this same test the percentage of rust in G 40 and G 149 was 4.15 and 3.88, respectively, G 149 showing the least rust of any strain of *Triticum vulgare* tested in 1925.

³ LEVINE, M. N., and STARKMAN, E. C. [REPORT ON STEM RUST INFECTION OF WHEAT AND OATS GROWN AT DIFFERENT STATIONS IN THE UNITED STATES AND CANADA IN 1925.] 4 tables. [1920.] [Mimeographed].

TABLE 4.—Average percentage of stem rust on 16 varieties and strains of spring wheat grown in uniform rust nurseries at different stations in the United States and Canada in 1925 ^a

Class, variety, and cereal investigation number	Calculated percentage of infection						A average for all regions
	Region						
	Prairie	Great Plains	Great Lakes	South- west	South- east	East Canada	
Hard Red:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Haynes, 2874	54.8	25.2	18.9	5.1	0.1	T. ÷	23.95
Marquis, 3611	55.8	31.2	24.4	6.0	.3	T. —	26.60
Power, 3697	63.6	30.6	23.9	8.7	.3	T. ÷	28.71
Ruby, 6047	45.1	28.7	24.3	6.0	.3	2.0	23.32
Marquillo, 6887	18.5	11.7	8.6	0	T. —	0	9.80
Preston, 3081	68.5	35.7	22.1	7.6	.1	T. ÷	30.89
Kota, 5878	15.0	6.8	4.7	2.6	T. —	T. ÷	6.59
Progress, 6902	24.2	7.7	6.9	0	0	T. —	10.12
Kota×Marquis, 6808	11.0	3.9	4.4	T. —	0	T. ÷	4.92
Marquis×Kansas, 7370	52.7	26.7	16.6	0	T. —	T. ÷	24.93
Ceres, 6900	17.3	9.5	7.0	0	T. —	0	8.62
Parker×Minnesota, 2222	37.2	21.0	3.5	0		B. T.	20.45
Weighted average for Hard-Red group.	38.6	19.9	13.7	5.0	.1	.2	18.43
White:							
Quality, 6607	36.5	22.3	18.1	0	.3	.1	19.39
Little Club, 4066	66.9	33.2	27.1	14.2	.1	.2	31.21
Dicklow×Sevier, G 40	9.7	1.8	1.0	0		0	4.15
Dicklow×Sevier, G 140	9.7	1.2	.5	0		0	3.88
Weighted average for White group.	30.7	14.6	12.5	8.9	.2	1	16.06

^a From mimeographed report of Levine and Stakman for the crop season of 1925. LEVINE, M. N., and STAKMAN, E. C. Op. cit.

GENETIC STUDY

Inasmuch as the Dicklow×Sevier cross proved extremely promising from the standpoint of wheat improvement, a cross between a pure line of Federation and a pure line of Sevier (Sevier 60) was used for a genetic study of spike density and awns. Observations were also made on the length and number of culms, on squareheadedness, and (in F_2) on thickness of neck, i. e., the diameter of culm measured just below the spike.

The cross was made in 1922 at the Utah Agricultural Experiment Station, Logan Utah. The F_1 plants were grown at Logan in 1923 and the F_2 plants at Logan in 1924. The writer entered the University of Minnesota in June, 1924, and after harvest, shipped the plants to University Farm, St. Paul, the plants having been pulled by the roots and packed carefully.

The hybrid plants were in 8 families, 5 of one cross (Sevier 60 × Federation) being numbered consecutively from 1 to 5. Family 4 was the largest and most uniformly developed family, and for this reason was chosen for the most detailed study. Observations were taken on families 1 to 3 in a somewhat, but not exactly, similar manner. Families 6, 7, and 8 were of another cross, Sevier G 101 × Federation.

METHOD OF TAKING OBSERVATIONS AND STUDYING DATA

The lengths of the culms were determined by placing the roots against a footboard nailed perpendicular to another board laid flat on a table. Centimeters were marked on the table board. This permitted a rapid reading of the length of the culm to the base of the spike. The length of the longest culm was read first and that of each succeeding shorter one in order.

The plants were classified with respect to awn types into groups 1, 2, 3, and 4. The plants in group 1 had only short beak awns, with an occasional apical awn. The intention was that this class should be comparable with Federation. Group 2 consisted of plants with intermediate awns, as nearly as possible like those of the F_1 plants. Group 4 was fully awned, as is Sevier, whereas group 3 was part way between groups 2 and 4. In group 3 the awns varied considerably in length, the apical awns were full length in some cases, but toward the base the awns were very short. There was so much variation in length of awn, especially in groups 2 and 3, that definite separation was difficult. Spikes showing the range of each awn class were kept constantly at hand to assist in classifying any given plant.

The length of the rachis in millimeters was taken from the node at the base of the spike to the base of the uppermost spikelet. The number of spikelets attached to the upper third and the number attached to the middle third of the rachis were counted. The ratio between these became the index of squareheadedness. Thickness of neck was measured on a leading culm with vernier calipers to the nearest tenth of a millimeter. Spike density was ascertained by measuring in millimeters on the spike of the same leading culm the length of that part of the rachis that extended from the base of one spikelet to the base of the sixth one above, i. e., the length of 10 rachis internodes. Care was taken to avoid the internodes near the base and those near the summit of the spike lest these should vary in length from those near the middle.

For the F_3 generation, which was grown at Logan, Utah, in 1925, random selections were made from plants which were represented on certain sections of the spike-density curve of family 4. Twelve sections on the curve (see fig. 3, top) were selected for study, and the first six plants, as they occurred in the record book, were taken as a random sample of that section of the curve. These were sown in the order in which they occurred in the record, beginning with the plants represented by the more dense end of the curve.

The F_3 generation lines of family 4 were sown in rows 1 foot apart and 12 feet long, about 50 kernels to the row, which resulted in about 40 mature plants. Each parent was sown in a similar manner in every fifth and sixth row. In families 5 and 6 all the plants were sown, 10 progeny rows being followed by 1 row of each parent.

In family 4 the same data were collected in F_3 as in F_2 , save that the thickness of neck was not measured. In family 5 only the awn data and the length of 10 rachis internodes were obtained, and in family 6 only the awn data.

The F_2 data for each plant were listed on a separate card, and each character was studied by itself. Afterward, a correlated study of the most important characters was made by calculating the coefficient of correlation (r) and the correlation ratio (η).

AWNS

In the cross between a pure line of Federation \times Sevier 60, F_2 families 1 to 5, inclusive, were classified for the four awn types previously described. Figure 2 shows the range in awn length for each of the four awn classes. The number of plants in each awn group in the five F_2 families is shown in Table 5.



FIG. 2.—The four classes of awns in the Federation \times Sevier cross. On the extreme right are the two parents, Federation (above) and Sevier (below). (Above, left to right). Awn class 1 (awnless); awn class 2 (short awn tips); awn class 3 (short-tip awns in lower half of spike and part-length awns in upper half); awn class 4 (fully awned). (Below, left to right). Awn class 4; awn class 3; awn class 2, and awn class 1.

TABLE 5.—Classification of five families of F_2 plants according to four awn classes. (Sevier 60×Federation)^a

Family	Number of plants in awn classes			
	1 Awn- less	2 Short awn tips	3 Awns short at base of spike but longer at apex	4 Fully awned
1	46	194	52	56
2	38	90	21	23
3	41	63	24	29
4	91	192	41	69
5	74	106	18	50

^a See fig. 2 for illustration of awn classes.

In F_3 there were 74 separate progenies of F_2 plants chosen at random from 12 selected sections of the spike-density curve of family 4. (See fig. 3.) Separate F_3 progenies of all the F_2 plants in families 5 and 6 were grown. There were 248 F_3 families in family 6 and 245 in family 5.

In family 4, the 74 F_3 families consisted of progenies of 14 F_2 plants classified as having awns 1, 37 plants classified as having awns 2, 7 as awns 3, and 16 as awns 4. In order to show the nature of awn behavior in the F_3 families, the detailed awn data for family 4 are given in Table 6.

TABLE 6.— F_3 breeding behavior of plants classified in F_2 into 4 classes on the basis of awn characters

[Family 4; grown in 1925 at Logan, Utah]

Classified when planted as being in awn class	Number of plants in awn classes—				Classified when planted as being in awn class	Number of plants in awn classes—			
	1	2	3	4		1	2	3	4
1	35	5			2	1	28		11
1	13	17	3	6	2	25	8	7	
1		40			2	4	16	12	8
1	35	4	1		2	11	17	6	8
1	24	14	2		2	3	25	6	6
1	20	19	1		2	8	9	4	7
1	7	14	19		2		12	14	14
1	13	16	10	1	2	9	19	4	7
1	28	11			2	1	20	10	9
1	23	4	12		2	25	11		
1	33				2			40	
1	19	21			2	6	11	16	7
1	26				2	34	2	4	
1	27				2		21	8	11
2	17	7			2				
2	1	28	5	11	3	14	15	11	
2		18	1	10	3		7	33	
2		18	4	3	3	14	18	7	
2	1	20	6	11	3		39		
2	27	7		6	3	9	21	10	1
2	21	6	3	9	3	35	4		
2	27	5	8		3	4	6		
2	16	6	4	10	4				36
2	8	28		9	4				36
2	11	2	6		4			1	
2	30	4	6		4				40
2	17	8	5	10	4				40
2	11	15	6	8	4		2	7	31
2	29	6	5		4				31
2		40			4				34
2	11	7	7	10	4				35
2	40				4		1	3	36
2	11	8	15	6	4		6	9	25
2	4	11	14	11	4				38
2	1	33	6		4		5	13	22
2	18	12	9	1	4			9	31
2	10	12	8	10	4			21	19
2		17	8	4	4				34

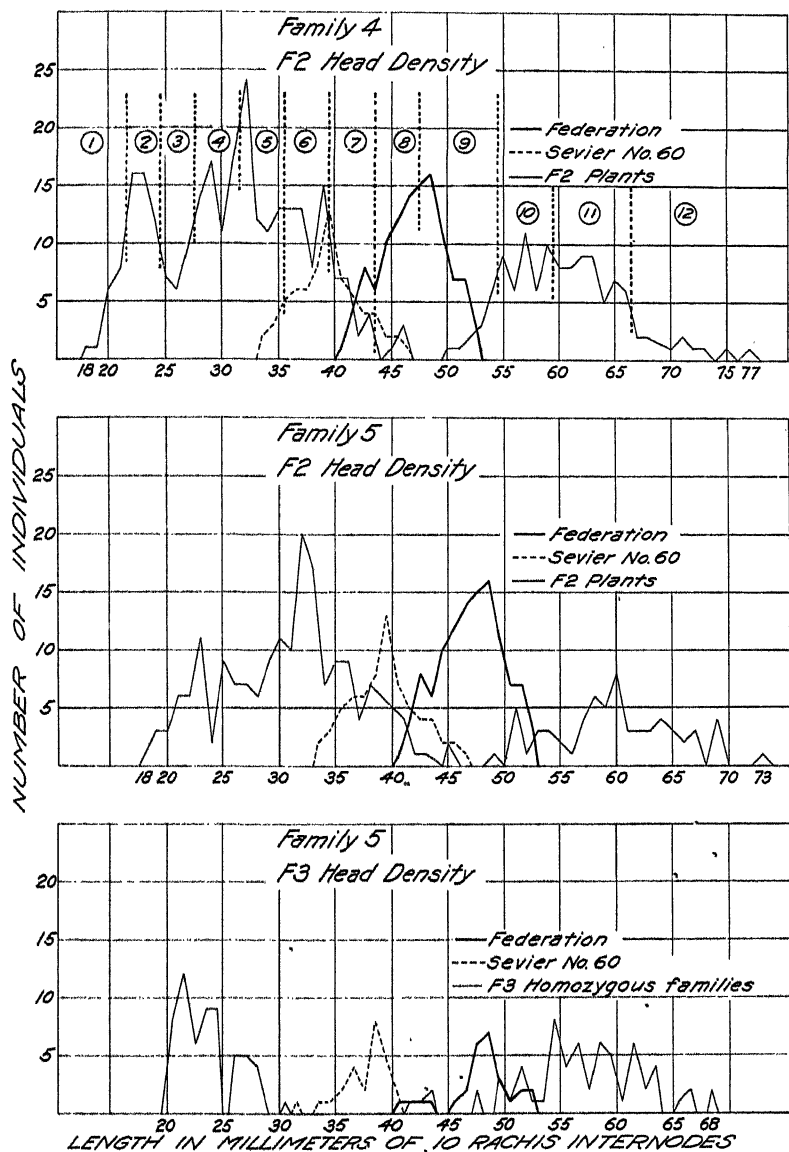


FIG. 3.—Spike density curves of the Federation and the Sevier parents and of F_2 and F_3 families. (Top) F_2 spike density curve of family 4, with curves of parental spike density superimposed. Vertical dotted lines divide the F_2 curve into 12 sections; 6 or 7 of the plants which each section represents were chosen at random to be used as mother plants of F_3 progenies. (Middle) F_2 spike density curve of family 5, with the curves of parental spike density superimposed. Grain of all the F_2 plants of family 5 was sown for F_3 progenies. (Bottom) Curve of mean spike density of homozygous F_3 families, with the curves of mean density of parental rows superimposed. All heterozygous families are omitted.

The F_3 showed the following behavior for awn classes:

Number of F_3 families breeding true for—		Number of F_3 families segregating for—	
Awns 1.....	4	Awns 1, 2.....	4
Awns 2.....	2	Awns 1, 2, 3.....	12
Awns 3.....	2	Awns 1, 2, 3, 4.....	25
Awns 4.....	7	Awns 2, 3, 4 (or 2, 4).....	12
		Awns 3, 4.....	6

In family 5, the seed of 247 F_2 plants was sown, one being omitted because of too few kernels. The plants of each F_3 family were classified according to awns. A summary of the data is given below:

Number of F_3 families breeding true for—		Number of F_3 families segregating for—	
Awns 1.....	18	Awns 1, 2.....	41
Awns 2.....	7	Awns 1, 2, 3.....	17
Awns 3.....	4	Awns 1, 2, 3, 4.....	66
Awns 4.....	18	Awns 2, 3, 4 (or 2, 4).....	49
		Awns 3, 4.....	7

In family 6, an F_3 progeny of every F_2 plant was likewise grown, each in a single row. The 40 or so plants in each row were classified and the data summarized by families, as follows:

Number of F_3 families breeding true for—		Number of F_3 families segregating for—	
Awns 1.....	29	Awns 1, 2.....	57
Awns 2.....	7	Awns 1, 2, 3.....	4
Awns 3.....	0	Awns 1, 2, 3, 4.....	70
Awns 4.....	21	Awns 2, 3, 4 (or 2, 4).....	46
		Awns 3, 4.....	11

An examination of the summaries of awn behavior shows that in the F_2 generation of families 4 and 5 there occurred 9 genotypes of plants according to awn-breeding behavior in F_3 . Four of these bred true and 5 segregated each in a distinct manner. In family 6 there were 8 genotypes, no F_3 family being found that bred true to awns 3.

On a basis of total F_3 families, the ratios of families that bred true for awns in various ways suggest a two-factor difference for awns. A strictly independent segregation would give approximately equal numbers in each of the four classes that bred true. There are, however, uniformly greater numbers in awn classes 1 and 4—the parental types—than in 2 and 3. This, and the distribution in the segregating classes, suggested linkage. It was found that as good fits as could be expected were obtained when the two factors for awns were considered to be so linked as to produce gametes in the proportion of 1.8 : 1 : 1 : 1.8.

Let us designate full awns, such as occur in the Sevier parent, by $AA TT$ (awn class 4); long apical awns but short lateral awns (awn class 3) by $AA tt$; short but well developed tip awns (awn class 2) by $aa TT$; and awnlessness such as occurs in the Federation parent by $aa tt$ (awn class 1). In strictly independent inheritance the four gametes AT , At , aT , and at would be produced in equal numbers.

In the degree of linkage here suggested, the gametes are produced 1.8 AT :1.0 At :1.0 aT :1.8 at . On this hypothesis the number and sort of gametes produced in F_1 and the number and sort of zygotes which would be expected in F_2 are given in Table 7.

TABLE 7. — *Theoretical number and sort of gametes produced by F_1 plants; theoretical number and sort of zygotic combinations resulting in F_2*

	1.8 $A T$	1.0 $A t$	1.0 $a T$	1.8 $a t$
1.8 AT	3.24 $AA TT$	1.8 $AA Tt$	1.8 $Aa TT$	3.24 $Aa Tt$
1.0 At	1.8 $AA Tt$	1.0 $AA tt$	1.0 $Aa Tt$	1.8 $Aa tt$
1.0 aT	1.8 $Aa TT$	1.0 $Aa Tt$	1.0 $aa TT$	1.8 $aa Tt$
1.8 at	3.24 $Aa Tt$	1.8 $Aa tt$	1.8 $aa tt$	3.24 $aa tt$

- | | | |
|--------------------|--|--------------------|
| (1) 3.24 $AA TT$. | | (6) 3.6 $Aa tt$. |
| (2) 3.6 $AA Tt$. | | (7) 3.6 $aa Tt$. |
| (3) 3.6 $Aa TT$. | | (8) 1.0 $aa TT$. |
| (4) 8.48 $Aa Tt$. | | (9) 3.24 $aa tt$. |
| (5) 1.0 $AA tt$. | | |

The expected breeding nature of the 9 genotypes is indicated here:

- | | | |
|------------------------------------|--|------------------------------|
| (1) Breed true for awns 4. | | (6) Segregate for awns 1, 2. |
| (2) Segregate for awns 3, 4. | | (7) Segregate for awns 1, 2. |
| (3) Segregate for awns 2, 3, 4. | | (8) Breed true for awns 2. |
| (4) Segregate for awns 1, 2, 3, 4. | | (9) Breed true for awns 1. |
| (5) Breed true for awns 3. | | |

Of the 9 sorts of genotypes (1), (5), (8), and (9), are the true-breeding forms for awn classes 4, 3, 2, and 1, respectively. Genotype (4) is of the same composition as the F_1 and its phenotype essentially like awns 2. Genotypes (2) and (3) are each homozygous for one dominant factor and heterozygous for the other, (2) being heterozygous for Tt and (3) for Aa . The progeny of these two groups of plants apparently have a range in awn length that extends from class 3 to class 4. Since these two types of segregates could not be satisfactorily separated in F_3 they were placed together in one class. Genotypes (6) and (7) differ only in that (6) is homozygous recessive for tt and heterozygous for Aa , whereas (7) is homozygous recessive for aa and heterozygous for Tt . These two groups were also deemed nonseparable and were therefore placed in one group.

The F_2 genotypes of families 4, 5, and 6 were determined by the F_3 breeding behavior, and the F_2 genotypes were classified for awns on the basis of F_3 breeding behavior. Families 4 and 5 were from crosses of the same pure lines, but family 6 was from a cross of the same pure line of Federation by a different line of Sevier. Since family 5 consisted in F_3 of progenies of all the F_2 plants, it was studied first. Its closeness of fit to the calculated expectancy on the basis of linkage, with 35 per cent crossing over, is shown in Table 8, where the calculated expectancy, the observed numbers, X^2 , and P are given. Similar data and the closeness of fit for families 4 and 6 are given in Tables 9 and 10, respectively.

TABLE 8.—Calculated (*C*) and observed (*O*) numbers of plants in each awn group of *F*₂ genotypes as determined by the *F*₃ breeding behavior

[Family 5; grown in 1925 at Logan, Utah]

Genotype	C	O	C-O	(C-O) ²	(C-O) ² C
<i>AA TT</i>	23.28	18	5.28	27.8784	1.1975
<i>AA Tt</i>	51.89	56	-4.11	16.8921	.3255
<i>Aa TT</i>	61.12	66	-4.88	23.8144	.3807
<i>AA tt</i>	7.21	4	3.21	10.3041	1.4291
<i>Aa tt</i>	51.89	58	-6.11	37.3321	.7191
<i>aa TT</i>	7.21	7	-0.21	.0441	.0061
<i>aa tt</i>	23.28	18	5.28	27.8784	1.1975
Total.....	226	227	<i>P</i> =.5118	<i>X</i> ² =5.2656	

TABLE 9.—Calculated (*C*) and observed (*O*) numbers of plants in each awn group of *F*₂ genotypes as determined by the *F*₃ breeding behavior

[Family 4; grown in 1925 at Logan, Utah]

Genotype	C	O	C-O	(C-O) ²	(C-O) ² C
<i>AA TT</i>	7.65	7	0.65	0.4225	0.0552
<i>AA Tt</i>	16.99	18	-1.01	1.0201	.0600
<i>Aa TT</i>	20.01	25	-4.99	24.9001	1.2444
<i>AA tt</i>	2.36	2	0.36	.1296	.0549
<i>Aa tt</i>	16.99	16	.99	.9801	.0577
<i>aa TT</i>	2.36	2	.36	.1296	.0540
<i>aa tt</i>	7.65	4	3.65	13.3225	1.7415
Total.....	74	74	<i>P</i> =0.7734	<i>X</i> ² =3.2686	

TABLE 10.—Calculated (*C*) and observed (*O*) number of plants in each awn group of *F*₂ genotypes as determined by the *F*₃ breeding behavior

[Family 6; grown in 1925 at Logan, Utah]

Genotype	C	O	C-O	(C-O) ²	(C-O) ² C
<i>AA TT</i>	25.31	21	4.31	18.5761	0.7339
<i>AA Tt</i>	56.25	57	-0.75	.5625	.0100
<i>Aa TT</i>	66.25	70	-3.75	14.0625	.2123
<i>AA tt</i>	7.81	0	7.81	60.9961	7.8100
<i>Aa tt</i>	56.25	61	-4.75	22.5625	.4011
<i>aa TT</i>	7.81	7	0.81	.6561	.0840
<i>aa tt</i>	25.31	29	-3.69	13.6161	.5380
Total.....	245	245	<i>P</i> =.1349	<i>X</i> ² =9.7893	

Tables 8 and 9 show that families 4 and 5 are very close fits to the calculated expectation on the hypothesis of two linked factors with a crossing over of 35 per cent. The value of *P* for family 5 was 0.5118, which shows that in 51 cases out of 100 a worse fit might be expected

from chance alone. Family 4 gave a little higher value for P , indicating another almost perfect fit. In family 6, however, P was only 0.1349, which indicates that, owing to random sampling, a worse result might be expected once in 7 or 8 trials.

It may therefore be safely concluded that the F_2 genotypes as classified by the F_3 breeding behavior rather highly substantiates the presence of two factors for awns, both in the same chromosome, with crossing over to the extent of about 35 per cent.

Three things must be done, however, to establish definitely the correctness of the hypothesis. (1) The linkage must be tested. If the true-breeding forms for awns 2 and 3 were crossed, the linkage could be tested, since the factors would then be linked in the opposite way, i. e., in a repulsion series. The expected gametic ratio would then be 1.0 AT : 1.8 At : 1.8 aT : 1.0 at . A larger number of forms breeding true for awn classes 2 and 3 than for awn classes 1 and 4 would be expected. (2) A back-cross between the F_1 and the parental homozygous double recessive must be made. This would show in the next generation the ratio of gametes produced by the F_1 plant. (3) A cross between the truebreeding forms of awns 2 and 3 and the double recessive Federation parent must be made. This would demonstrate the correctness of a one-factor difference between each of these and Federation. In this third test it would also be interesting to know the behavior of the progeny of the same crosses with the double homozygous dominant as well as with the recessive.

SPIKE DENSITY

All of the 8 F_1 plants produced semiclubbed spikes, i. e., though they were not measured for internode length, they were visibly of intermediate spike density. The F_2 showed a wide and striking variation in average length of rachis internodes, some segregates being far more compact and others far more lax than was the case with either parent.

Table 11 gives the data on length of internode for families 4 and 5 and for a small number of plants of each parent grown in a single row in the same part of the field as the F_2 families.

In F_3 , 74 plants of F_2 were selected at random from various parts of the frequency curve for spike density of family 4 (see fig. 3, top). The F_3 planting plan was so arranged that each four hybrid F_3 families had a row of Federation and one of Sevier on each side, i. e., there was a row of each parent then four hybrid rows followed by a row of each parent and these by four hybrid families until all were seeded. There were about 40 plants in each row, a leading spike of each of which was measured for density. The same procedure was followed for family 5, save that progeny of all the F_2 plants were sown and the parents occurred only after each 10 F_3 rows. Two hundred and forty-seven F_3 progenies of family 5 were tested for spike density. (See fig. 3, middle.)

TABLE 11.—Number of plants of Sevier, of Federation, and of the F_2 hybrids between Sevier and Federation grouped into classes according to the average length of rachis internodes (the length in millimeters of 10 internodes was measured in the middle of the head of one main culm on each plant and the length of one internode obtained by pointing off 1 decimal place)

[Grown in 1921 at Logan, Utah]

Strain	Number of plants in classes of spike density													
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25	7.75	
Federation						29	68	17						
Sevier				5	38	22	3							
Family 4		58	54	65	62	20	4	13	42	39	17	7	2	
Family 5	4	28	38	65	35	11	3	12	18	21	12	1		

To determine how soil heterogeneity affected the variability of the mean internode lengths of Sevier and Federation, coefficients of correlation were calculated for the adjacent rows of Sevier and Federation 1 foot apart, for the rows of Sevier 6 feet apart, and for rows of Federation 6 feet apart. The same calculations were made for family 5. In family 5, however, the two Sevier rows and the two Federation rows were 12 feet apart. The coefficients of correlation (r) are given in Table 12.

TABLE 12.—Correlation coefficients of spike density between rows of Sevier and Federation 1 foot apart and between each pair of two rows of Sevier and Federation 6 feet apart, in family 4, and 12 feet apart in family 5

[Grown in 1925 at Logan, Utah]

Strains	Coefficient of correlation in family 4	Coefficient of correlation in family 5
Federation X Sevier.....	+0.421	+0.579
Sevier X Sevier.....	+.208	-.032
Federation X Federation.....	-.104	-.004

Table 12 shows that the spike density of adjacent rows is definitely correlated, but that rows 6 feet apart and 10 feet apart do not vary significantly in the same direction. The results indicate the extent of variation in spike density which results from the soil heterogeneity of the field. The amount to which total variability in mean internode length is dependent upon soil heterogeneity can be determined by substituting r in the equation $v = 1 - \sqrt{1 - r^2}$, where v = variation in percentage. In these tests the variation is found to be from about 10 to 15 per cent.

The mean spike density of each F_2 family was obtained, and the coefficients of variability (C. V.) of the individual rows were calculated. A study of this constant for each family showed whether the family was breeding essentially true or was segregating. Table 13 shows the mean spike density classes and the coefficient of variability classes of rows of each parent and of three groups of F_2 hybrids, namely, those breeding essentially true for dense spikes; those

segregating for spike density; and those breeding essentially true for lax spikes. Table 14 gives similar data for family 5.

The parent rows sown with the hybrids of family 4 had coefficients of variability (C. V.) ranging from 4.50 per cent to 9.34 per cent in the case of Federation, with a mean coefficient of variability of 7.40 per cent for the 24 rows. In the case of Sevier 60 the range was from 4.50 to 15.41 per cent and the mean was 7.40 per cent.

For the homozygous dense group the range in the calculated coefficients of variability was from 7.46 to 13.14 per cent, with a mean of 10.34 per cent. In the heterozygous group the range in the calculated coefficients of variability was from 24.82 to 52.30 percent, with a mean of 33.27 per cent. The mean for the homozygous lax group was 7.45 per cent, with a range from 4.55 to 10.71 per cent. In family 5 the ranges and the means of the coefficients of variability were essentially the same, the means being 6.87 per cent for Federation, 7.16 per cent for Sevier 60, 9.90 per cent for the homozygous dense segregates, 33.70 per cent for the heterozygous, and 7.47 per cent for the homozygous lax segregates.

TABLE 13.—*Spike density classes of the means of Sevier and Federation parental rows and of the F₂ families and coefficients of variability classes (C. V.) of the individual rows of the Sevier and Federation parents and of three groups of hybrids—(1) those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 4; grown in 1925 at Logan, Utah]

Strain	Number of plants in spike-density classes											Total	C. V. classes
	1. 75	2. 25	2. 75	3. 25	3. 75	4. 25	4. 75	5. 25	5. 75	6. 25	6. 75		
Federation.....	{						12	3				15	6
							4	1				5	9
Total.....							16	4				Mean.	6. 87
Sevier 60.....	{				13	1						14	6
					4							4	9
					1							1	12
					1							1	15
Total.....					19	1						Mean.	7. 40
Homozygous dense.....	{	2										2	6
	3	4	1									8	9
	2	5	2									9	12
Total.....	5	11	3									Mean.	10. 34
Heterozygous.....	{					1						1	24
						4	1					5	27
					1	3	3					7	30
					1	1	1					3	33
			1	1	1	1	1					4	36
					3	1						4	39
				1								1	42
												0	45
												0	48
					1							1	51
Total.....			1	4	13	8						Mean.	33. 27
Homozygous lax.....	{						1	2	8	5	1	17	6
								3	6	1	1	11	9
										1		1	12
Total.....							1	5	14	7	2	Mean.	7. 45

TABLE 14.—Spike density classes of the means of Federation and Sevier parental rows and of the F_3 families and coefficients of variability classes (C. V.) of the individual rows of the Sevier and the Federation parents and of three groups of F_3 hybrid families—(1) those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes

[Family 5; grown in 1925 at Logan, Utah]

Strain	Number of means in spike-density classes											Total	C. V. classes
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75		
Federation.....	{					1	14	1				16	6
						1	4	3				8	9
Total.....						2	18	4				Mean.	6.87
Sevier 60.....	{			2	1	14	1					1	3
					4							17	6
				1								4	9
					1							1	12
Total.....				3	20	1						Mean.	7.16
Homozygous dense.	{	1	2	3								5	6
		1	27	8								36	9
		1	11	3								15	12
		1		1								2	15
Total.....		3	41	15								Mean.	9.90
Heterozygous.....	{			1	1		1					1	21
				3	6	4	1					3	24
			1	8	16	6						13	27
				6	12	6						31	30
			1	2	11	7						24	33
			2	5	8	1						21	36
			6	3								16	39
			1									9	42
Total.....			6	34	57	24	2					Mean.	33.70
Homozygous lax.....	{					1	1	9	12	5	4	1	3
						3	4	8	0	6	1	32	6
												31	9
								1				0	12
Total.....						4	5	18	22	11	5	Mean.	7.47

The calculated coefficient of variability for each of the F_3 families shows which are breeding relatively true and which are segregating for spike density. The difference between mean coefficients of variability of 10 per cent for those breeding true and 33 per cent for those segregating is so great, as to make certain which rows are homozygous or nearly so.

In family 5 grain of each of the F_2 plants was sown. Of the 247 F_3 families, 59 bred true for dense, 64 for lax, and 123 segregated for density (see fig. 4). Table 15 gives the calculation for closeness of fit on a one-factor difference for spike density.

TABLE 15.—*Closeness of fit of three groups of F_3 families compared with the 1:2:1 segregation*

[Family 5; grown in 1925 at Logan, Utah]

Group	C	O	C-O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous dense	61.75	59	2.75	7.5625	0.1224
Heterozygous	123.50	123	.50	.2500	.0020
Homozygous lax	61.75	64	2.25	5.0625	.0820
$X^2 = 0.2064$					

Since X^2 is less than 1, the probability is very great that the proposed hypothesis is essentially correct. Family 5, in which 247 F_3 families were measured, shows a very close approximation to a

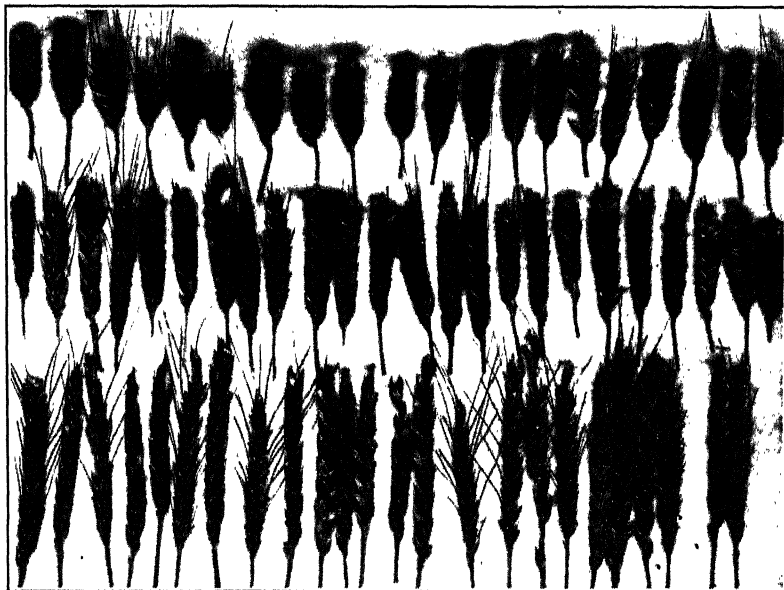


FIG. 4.—Single spikes of each F_3 plants of family 4 in the cross between pure lines of Federation and Sevier. When tested in F_3 all the forms in the top row proved homozygous for dense spikes; those in the bottom row homozygous for lax spikes, and those in the middle row heterozygous for density. There was a very close approximation to a ratio of 1 dense: 2 heterozygous: 1 lax, indicating one major factor difference for spike density. There are in each major group smaller inherited differences in spike density, which indicate that in addition to the one major factor there is a series of minor factors involved in the inheritance of spike density.

1:2:1 ratio. This suggests that one main factor is involved in the inheritance of spike density.

That spike density in the Sevier \times Federation cross has involved, in addition to one main factor, several minor factors is evident from the fact that one parent was not recovered at all. By referring to Figure 3 (bottom), it can be seen, however, that the least dense family of the homozygous dense segregates is so near to the most dense row of Sevier that if the 10. to 15 per cent variation due to soil heterogeneity be allowed for, it might be considered that there was one F_3 family in which the density of Sevier was recovered. Since there were 247 F_3 families in family 5 and 74 in family 4, the number of segregates breeding true to the spike density of the Sevier parent is so small as to make clear that several minor factors are involved.

This is also borne out by the range of mean densities of the homozygous dense and homozygous lax families. A summary of the range of densities in the parents and in the F_3 families is given in Table 16.

TABLE 16.—*The range of mean spike densities and the mean of mean spike densities of Federation and Sevier rows and of three groups of F_3 families and coefficients of variability (C. V.) of the mean spike densities of Federation and Sevier rows and of F_3 families*

Parent or segregates	Range of spike densities of Federation and Sevier parents and of F_3 families		Mean spike density of parents and F_3 families		C. V. of mean spike densities of parents and F_3 families	
	Family 4	Family 5	Family 4	Family 5	Family 4	Family 5
Federation.....	4, 567-5, 251	4, 055-5, 237	4, 880	4, 752	3.28	5.47
Sevier.....	35, 92-40, 12	3, 135-4, 022	37, 58	37, 21	2.45	5.47
Homozygous dense.....	1, 945-2, 782	1, 952-2, 825	2, 265	2, 325	10.50	16.67
Heterozygous.....	2, 925-4, 495	2, 437-4, 502	3, 781	3, 688	10.63	10.40
Homozygous lax.....	4, 732-6, 911	4, 110-6, 794	5, 869	5, 600	7.10	10.63

The coefficients of variability of the mean densities of the 20 parent rows sown in the family 4 plot were only 3.28 per cent for Federation and 2.45 per cent for Sevier 60 as compared with 10.50 per cent for the homozygous F_3 families and with 7.10 per cent for the homozygous lax families. The same figures for family 5 are all noticeably larger, probably because of greater soil heterogeneity resulting from the fact that the larger number of rows covered about three times as large an area of land. The figures for family 5 are 5.47, 5.47, 16.67, and 10.63 per cent, respectively.

On an average, the variability of the means of the homozygous F_3 families is about 2 to 3 times as great as that of the 20 to 24 parent rows planted at frequent intervals. This, added to the fact that there is a series of small steps throughout the range of variation in mean spike densities, points rather strongly to a series of minor factors which modify the expression of the one major factor for spike density.

As yet there has been found no genetic hypothesis that will explain the fact of wide transgressive segregation of spike density in both directions and still account for the very infrequent recovery of the spike density of one of the parents.

SQUAREHEADEDNESS

The squareheadedness of the 74 F_3 families of family 4 which were grown was obtained by calculating the ratio of the number of rachis internodes in the middle third of the rachis to the number in the upper third. This calculation was made for 20 rows of about 40 plants each of the two parents as well as for the F_3 families. The frequency with which rows of the Federation and the Sevier parents and of the F_3 families occurred when put in classes according to mean squareheadedness and in relation to their calculated coefficients of variability is given in Table 17. The means of the squareheadedness of Federation ranged from 0.99 to 1.17, with a range in the coefficients of variability of from 9.59 to 19.43 per cent, the mean being 14.84 per cent. The range in the mean squareheadedness of the rows of Sevier 60 was from 0.77 to 1.08. The range in the coefficients of variability was from 10.85 to 21.88 per cent, with a mean of 15.30 per cent. The mean squareheadedness of the F_3 families ranged from 0.90 to 1.30. Their smallest coefficient of variability was 10.13 per cent and the largest 29.24 per cent. The mean was 15.81 per cent.

TABLE 17. --Frequency distribution of rows of the Federation and the Sevier parents and of 74 F₃ families arranged in classes according to the mean squareheadedness and according to the size of the coefficients of variability

[Family 4; grown in 1925 at Logan, Utah]

Strain	Distribution of rows according to classes of squareheadedness																			Total	C. V. classes	
	.78	.81	.84	.87	.90	.93	.96	.99	1.02	1.05	1.08	1.11	1.14	1.17	1.20	1.23	1.26	1.29				
Federation										1			2	2		1					1	9
									1	1	2			1	2						7	12
								1		1	2				1						7	15
															1						6	18
Total								1	1	3	4	2	4	5							Mean	14.84
Sevier 60				2			3	1	1												5	12
			1	2		1	2			1	1										7	15
	1	1		2																	4	18
																					4	21
Total	1	1		5	2	1	6	1	1	1	1										Mean	15.40
F ₃ families									1												1	9
					1				2	1	4	5					1				14	12
				3		2	3	6	5	5	3	3	1	1	1	1	1				34	15
					1	3	2	2	3	3	1	1	1			1					18	18
									1	1					1						4	21
										1	1										1	24
												1									1	27
											1										1	30
Total				3	2	5	5	12	10	15	10	4	2	2	3				1		Mean	15.81

The distribution of the rows of each parent indicates a genetic difference in squareheadedness between the Federation and the Sevier parents. The variations in squareheadedness are so great as to make impossible an accurate study of the inheritance of this character. Though the F₃ families have a somewhat wider absolute range of squareheadedness and of coefficients of variability, the distribution of the F₃ families into each group does not give much greater calculated coefficients of variability than those for the parents. No correlation for squareheadedness was found between F₂ plants and F₃ families.

TALLEST CULM

The plants in the F₃ families of family 4, together with the plants from the respective rows of each parent, were measured in order to ascertain the length of the tallest culm. The average height of the shortest row of Federation was 92.2 cm. and that of the tallest 106.3 cm., the mean height being 99.8 cm. For Sevier 60 the average height of the shortest row was 112.8 cm., and that of the tallest 126.3 cm., with a mean height of 119.1 cm. For the 74 F₃ families the mean height of plants in the shortest family was 99.0 cm. and that of the tallest family 135.3 cm., with a mean height of 111.4 cm.

The frequency distribution of the means of both parents and of the means of the 74 F₃ families when grouped in classes according to the heights of plants and the coefficients of variability is shown in Table 18. From the table it is apparent that there was a distinct difference in the length of the tallest culms of the two parents. The means of culm lengths in F₃ families were more variable than those of either parent. This is shown by the fact that the coefficient of variability of the means of the F₃ lines is 5.29 per cent, whereas the mean coefficient of variability of Federation is 4.54 per cent, and that of Sevier 3.63 per cent.

TABLE 18.—Frequency distribution of rows of the Federation and the Sevier parents, and of 74 F_3 families arranged into classes according to height of the tallest culm in centimeters and according to the size of the coefficients of variability

[Family 4; grown in 1925 in Logan, Utah]

Strain	Rows in classes based on height of tallest culm															Total	C. V. classes
	93	96	99	102	105	108	111	114	117	120	123	126	129	132	135		
Federation			1		2											3	3
		2	3	3	1											9	4
		2	1	1												4	5
		1			1											2	6
	1		1													2	7
Total	1	5	6	4	4											Mean.	4.74
Sevier 60								1		1						1	2
								5	2	1	3	1				6	3
								1			2	2				12	4
																1	5
								7	2	3	5	3				Mean.	3.63
F_3 families					2	2	2	3	2	2	3				1	17	4
			1	5	6	7	3	3	1	1	1					27	5
		1	1	4	7	2	5	2								23	6
				1			2	1			1					5	7
					1											1	8
Total						1										0	9
																1	10
			2	1	13	16	13	12	7	3	6				1	Mean.	5.29

The distribution of the means of the parental rows in classes according to the average height of the plants in relation to coefficient of variability classes indicates a genetic difference between the Federation and the Sevier parents. The mean heights of the F_3 families range from a little more than the average height of the shortest row of Federation to somewhat more than the average height of the tallest row of Sevier.

These results indicate that there was segregation for this character, but that its nature can not be accurately determined from the data available.

CORRELATION STUDIES

A study was made of F_2 plants to find out whether certain plant characters were correlated. The observations on each plant were taken separately, and later the data relating to each plant were copied on separate cards. This permitted sorting the cards into groups according to the characters being correlated. Forty-nine tables were made in connection with the 8 families, for each of which the coefficient of correlation (r), the correlation ratio (η), and Blake-man's test for linearity were calculated. These constants are given in Table 19.

Spike density has been shown to be a definitely inherited character dependent upon one major factor. The first part of Table 19 gives the correlations of spike density with other F_2 plant characters.

In 4 families the correlation between spike density and the length of the longest culm was studied. The r 's are all small, partly — and partly +, and indicate an absence of correlation such as r will measure. On the other hand, the η 's all approach 0.2 to 0.3, which

figures are from 4.7 to 9 times the probable errors, and suggest that some sort of correlation exists. Since the values of three of the four Blakeman's tests for linearity are greater than 3 and the other one is greater than 2, the chances seem to be that there is a small correlation, with about 1 chance in 20 of its being linear.

TABLE 19.—Correlation coefficients (r), correlation ratios (η), their respective probable errors ($P. E.$), and Blakeman's test of linearity for various pairs of plant characters

[F_2 individual plants, grown in 1924 at Logan, Utah]

Characters related, and family	Number of plants	$r \pm P. E.$	$\frac{r}{P. E.}$	$\eta \pm P. E.$	$\frac{\eta}{P. E.}$	Blakeman's test
Spike density \times longest culm:						
Family 4	303	$-.050 \pm 0.34$	1.7	$0.241 \pm .032$	7.6	3.4325
Family 6	246	$-.027 \pm .043$.6	$.193 \pm .041$	4.7	2.2285
Family 7	301	$+.079 \pm .030$	2.0	$.315 \pm .035$	9.0	3.9177
Family 8	239	$-.128 \pm .043$	3.0	$.205 \pm .040$	7.4	3.0278
Spike density \times number of culms:						
Family 4	303	$-.108 \pm .034$	3.2	$.196 \pm .033$	5.9	2.4033
Family 5	248	$+.035 \pm .043$.8	$.223 \pm .041$	5.4	2.5673
Spike density \times total length of culms:						
Family 4	303	$-.043 \pm .034$	1.3	$.205 \pm .033$	6.2	2.9365
Spike density \times awn classes:						
Family 4	303	$-.020 \pm .034$.6	$.118 \pm .034$	3.5	1.7253
Family 5	248	$-.079 \pm .043$	1.8	$.228 \pm .041$	5.6	2.4996
Family 6	247	$-.035 \pm .043$.8	$.223 \pm .041$	5.7	2.6798
Family 7	301	$+.131 \pm .038$	3.4	$.150 \pm .038$	3.9	.9556
Family 8	239	$-.014 \pm .044$.3	$.211 \pm .042$	5.0	2.3518
Spike density \times thickness of neck:						
Family 5	248	$-.040 \pm .043$	1.1	$.355 \pm .037$	9.6	4.1151
Spike density \times square-headedness:						
Family 1	349	$-.545 \pm .025$	21.8	$.573 \pm .024$	23.9	.2527
Family 2	173	$-.502 \pm .038$	13.2	$.602 \pm .033$	18.2	3.2362
Family 3	156	$-.591 \pm .035$	16.9	$.628 \pm .033$	19.0	1.9611
Family 4	303	$-.542 \pm .024$	22.6	$.615 \pm .021$	29.3	4.2739
Family 5	248	$-.542 \pm .030$	18.1	$.570 \pm .028$	20.4	2.0510
Family 6	247	$-.515 \pm .032$	16.1	$.608 \pm .028$	21.7	3.7538
Family 7	301	$-.463 \pm .031$	14.9	$.578 \pm .026$	22.2	1.8081
Family 8	239	$-.503 \pm .033$	15.2	$.544 \pm .031$	17.5	2.3824
Awn classes \times spike shape (eye classification):						
Family 4	303	$+.042 \pm .034$	1.2	$.070 \pm .034$	2.1	.8141
Awn classes \times longest culm:						
Family 4	303	$-.038 \pm .034$	1.1	$.115 \pm .033$	3.5	2.1610
Family 6	247	$+.019 \pm .043$.4	$.205 \pm .041$	5.0	2.3815
Awn classes \times number of culms:						
Family 4	303	$+.112 \pm .034$	3.3	$.160 \pm .033$	4.8	1.6822
Family 6	247	$-.037 \pm .043$.9	$.217 \pm .041$	5.2	2.4865
Longest culm \times total length of culms:						
Family 4	303	$+.530 \pm .024$	22.1	$.504 \pm .023$	24.5	2.8304
Longest culm \times number of culms:						
Family 4	303	$+.340 \pm .030$	11.3	$.392 \pm .029$	13.5	2.8673
Square-headedness \times longest culm:						
Family 1	349	$-.054 \pm .036$	1.5	$.330 \pm .032$	10.3	4.5138
Family 2	173	$+.137 \pm .050$	2.7	$.306 \pm .046$	6.7	2.0740
Family 3	156	$+.240 \pm .051$	4.7	$.649 \pm .051$	20.9	5.6084
Family 4	303	$-.029 \pm .034$.8	$.208 \pm .033$	6.3	3.0247
Family 7	301	$-.035 \pm .039$.9	$.265 \pm .036$	7.4	3.3725
Family 8	239	$+.020 \pm .044$.5	$.331 \pm .039$	8.5	1.1775
Square-headedness \times thickness of neck:						
Family 1	349	$+.153 \pm .035$	4.4	$.339 \pm .032$	10.6	4.1853
Family 2	173	$+.116 \pm .051$	2.3	$.318 \pm .046$	7.0	2.3885
Family 3	156	$+.248 \pm .051$	4.9	$.301 \pm .050$	6.0	1.5564
Family 4	248	$+.212 \pm .041$	5.2	$.315 \pm .039$	8.1	2.7075
Family 5	247	$+.147 \pm .042$	3.5	$.330 \pm .038$	8.7	3.4382
Family 6	247	$+.146 \pm .038$	3.8	$.196 \pm .037$	5.3	1.6964
Family 7	301	$+.077 \pm .043$	1.8	$.199 \pm .042$	4.7	2.0849
Square-headedness \times awn classes:						
Family 1	349	$-.028 \pm .036$.8	$.133 \pm .036$	3.7	1.7943
Family 2	173	$-.063 \pm .051$	1.2	$.221 \pm .049$	4.5	2.0591
Family 3	156	$-.084 \pm .053$	1.6	$.269 \pm .050$	5.4	2.3738
Family 4	303	$-.110 \pm .034$	3.2	$.155 \pm .033$	4.7	1.5948
Family 5	248	$-.024 \pm .043$.6	$.166 \pm .042$	3.9	1.9235
Family 6	247	$-.020 \pm .043$.5	$.255 \pm .040$	6.3	2.9671
Family 7	301	$-.130 \pm .038$	3.7	$.414 \pm .032$	12.9	5.0088
Family 8	239	$-.011 \pm .044$.3	$.141 \pm .043$	3.3	1.5646

Spike density and number of culms seem to show a slight correlation as measured by the η 's, which are 0.196 and 0.223, values which are more than five times their probable errors. The two r 's are small and contradictory, and the two Blakeman's tests indicate that the chances are 1 in 4 that the correlation is linear.

The r for spike density correlated with total length of culm was -0.043 , scarcely larger than its probable error. The η is 0.205, which is 6 times its probable error. This apparently indicates a small correlation. Since the Blakeman's test is essentially 3, the chances are about 1 in 20 of its being linear.

The r 's for spike density and awn classes are small, some plus and some minus. The η 's vary from 0.1 to more than 0.2 and from three and five-tenths to more than five times their probable errors. The values of η are small, but seem to suggest the likelihood of a small correlation. The five Blakeman's tests vary from 0.9 to 2.6, values which suggest linearity. Such will be the case, however, when the η is very small even if r is essentially 0.

In the one correlation study of spike density and thickness of neck, r is too small to be significant, but η is 0.355, which is nine times its probable error. This is a fairly strong indication of a correlation. The fact that the value of Blakeman's test is more than 4 indicates that the chance of linearity is 1 in 142 trials. This suggests nonlinearity.

The coefficients of correlation of spike density \times squareheadedness are all above 0.5 and are from about 13 to more than 20 times the probable errors. The η 's are all 0.6 or nearly so and from 17 to 23 times the probable errors. Five of the eight Blakeman's tests are less than 3, and three are between 0.2 and 2, indicating linearity. All these figures seem to indicate a strong tendency of the more dense spikes to be more squareheaded.

Since, however, squareheadedness was so very greatly influenced by environment that the nature of its inheritance was not determined, it can not be safely said that even these large figures indicate linkage.

When awn classes and spike shape as determined by eye classification were correlated, r and η were both essentially 0, and neither was significant in the light of its probable error, indicating absence of correlation between these two characters.

In the correlation between awn classes and the number of culms, the η 's are about five times their probable errors and suggest some correlation, though one r is plus and the other minus. The values of the two Blakeman's tests for linearity are one less than 2 and the other more than 2. The chances are about 1 in 4 that there is linearity in the correlation.

In the correlation between awn classes and length of the longest culm in the two families represented, the r 's are very small and contradictory. One η is five times its probable error and the other one three and five-tenths times. These may indicate some correlation. The values of the two Blakeman's tests for linearity indicate that there is 1 chance in 5 or 6 of the correlation's being linear.

One would expect to find a high correlation between the length of the longest culm and the total culm length. Both r and η are greater than 0.5 and more than 20 times the probable error. It may be that

this figure is an additive result of plant vigor and of correlating two things one of which is part of the other.

In the one family studied, the length of the longest culm and the number of culms seem to be positively related since r is $+0.340$ and is 11 times its probable error; η is 0.392 and 13 times the probable error. Blakeman's test for linearity is less than 3 and also suggests linearity. It may be, however, that vigorous plants would tend to produce both more culms and longer ones. In so far as vigor and length of culm are due to the same genetic factor, even with the fair-sized constants, the correlation would be physiologic rather than genetic.

When squareheadedness is correlated with the length of the longest culm, with the thickness of the neck, and with awn classes, most of the r 's are small and the η 's fairly good sized and 3 to 20 times their probable errors. If squareheadedness were heritably a stable character, it is likely that constants of the magnitude obtained would indicate correlations. In view of the great influence of environment on the variability of squareheadedness in the material here used, it does not seem wise to ascribe much genetic importance to these figures.

SUMMARY

In a variety cross of Dicklow \times Sevier wheats, some F_3 and F_4 families considerably more compact of spike than either parent and some considerably more lax than either parent were found to breed true. In the nursery test some of the homozygous lines gave yields sufficiently higher than that of the high-yielding parent to appear promising from the standpoint of wheat improvement.

About 50 of the homozygous lines from the Dicklow \times Sevier cross were tested at University Farm, St. Paul, Minn., in 1924 for resistance to *Puccinia graminis tritici*. Two of the lines (G 40 and G 149) were found to be very resistant in the field. Seedling plants of these two lines and of both the parents were inoculated in the greenhouse with all the physiologic forms of black stem rust then available. The Dicklow parent was completely susceptible to every form tested. The Sevier parent was somewhat resistant. G 40 and G 149 proved resistant, G 149 was highly resistant to many forms and at least moderately resistant to all of the 19 forms available for testing.

In 1925, G 40 and G 149 were grown in the uniform rust nurseries in various parts of the United States and Canada and proved to be as resistant as the most highly resistant varieties of vulgare wheat known.

A detailed genetic study was made of a cross between pure lines of Federation and Sevier wheats, which were contrasted as follows:

Federation	Sevier
Awnless. Spike density intermediate. Slightly squareheaded. Short culms.	Awned. Spike density intermediate, but a little more dense than that of Federation. Not squareheaded. Long culms.

In F_2 a study was made of individual plants. Four classes of awns were defined, and each plant was classified by comparison with mounted specimens showing the range of variability for each class.

Measurements were taken of the length of the rachis, the length of 10 rachis internodes, the thickness of the neck just below the spike, and the length of the culms; and counts were made of the number of spikelet internodes in the middle third of the rachis and in the upper third (as a basis for calculating the ratio of squareheadedness) and of the number of culms.

In F_3 the F_2 genotypes were tested by the breeding behavior of F_3 families, each from a single F_2 plant. One row each of the Federation and the Sevier parents was sown among the F_3 families after every 4 hybrid rows in family 4 and after every 10 hybrid rows in families 5 and 6. Mother plants for the 74 F_3 progenies of family 4 were chosen at random from plants represented on each of the 12 sections of the spike-density curve of family 4. Grain of all the F_2 plants of families 5 and 6 was sown.

At harvest the F_3 progenies of family 4 were studied to determine awn classes spike density, squareheadedness, and length of culm. The 247 F_3 progenies of family 5 were studied to determine spike density and awn classes, and the awn classes of the 245 F_3 progenies of family 6 were determined.

Four homozygous classes of awns were obtained, one of each parental type and two intermediate types. In all three families the two parental classes were considerably more numerous. This fact suggested linkage, and when the observed proportions of homozygous and segregating progenies were studied by the closeness-of-fit method it was found that the hypothesis that there are two factors for awns located in the same chromosome and that there is 35 per cent crossing over explains the results.

The F_2 plants, as tested by the F_3 breeding behavior, gave a close approximation to a 1:2:1 ratio of dense, intermediate, and lax spikes. Approximately one-fourth of the total families had dense spikes and one-fourth had lax spikes. Both of these groups of families proved homozygous, and the intermediates all segregated.

In each of the three major groups there was a series of smaller differences in spike density, as is shown by the fact that the coefficients of variability of the means of the spike densities in the three major groups of F_3 families were considerably greater than were the coefficients of variability of the rows of either parent. The spike density of the Sevier parent was obtained again in not more than one of the 321 families tested and perhaps not all. Therefore, one major factor and a series of minor factors are involved in the inheritance of spike density in the Federation x Sevier cross.

The ratio of squareheadedness was found to be distinctly different in the Federation and in the Sevier parents. The ranges in the individual rows of the parents and in the F_3 progenies showed clearly an enormous variation in this character, due to environment. The coefficients of variability were approximately 15 per cent.

In order to find out the nature of inheritance of squareheadedness it would therefore be necessary to reduce variability due to environment by growing F_3 and F_4 families in replicate rows in various parts of the field adjacent to the parents.

The average height of Federation was distinctly less than the average height of Sevier. The F_3 families gave a range of mean heights about equal to the ranges of both parents combined. There

was definite evidence that segregation had taken place as regards height of plant, but the nature of the segregation could not be determined accurately. This could be studied by growing replicate rows of hybrids and parents in various parts of the field.

In correlation studies of F_2 plant characters, r , η , and Blakeman's test of linearity were calculated. Small but apparently significant correlations as judged by their probable errors were found between spike density and the length of the longest culm, between spike density and the number of culms, between spike density and the total culm length, between spike density and awn classes, between spike density and thickness of neck, between awn classes and the length of the longest culm, and between awn classes and the number of culms.

The odds were about 142 to 1 that the correlation between spike density and thickness of neck was nonlinear. In all the other cases the odds were rather small and suggest that the other correlations are a reasonable approach to linearity, as judged by the number of series calculated.

Squareheadedness correlated with spike density gave rather large r 's and η 's. When squareheadedness was correlated with awn classes the η 's were 3 to 12 times their probable errors. However, in view of the great variability of the squareheadedness character due to environment, no great genetic importance was attached to these correlations.

Rather large r 's and η 's were found in the correlation study between the length of the longest culm and total culm length and between length of the longest culm and the number of culms. These correlations were thought to be physiologic rather than genetic in their nature.

In view of the consistently significant correlations, as judged by their probable errors, between the two stable and definitely inherited characters of spike density and awn classes, it seems reasonable to conclude that there is a strong suggestion of linkage between the factors for these two characters.

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